THE USE OF 2*H*-AZIRIN-3-AMINES IN PEPTIDE SYNTHESIS: SYNTHESIS OF THE PROTECTED 6-16 SEGMENT OF THE PEPTAIBOL ZERVAMICIN II-2 AND TOTAL SYNTHESIS OF THE PEPTAIBOLS HYPOMUROCIN A1, A3 AND A5

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Meiner Mutter und Patrick in Dankbarkeit,
diese Arbeit ist euch gewidmet,

Meinem Vater,

Meinen deutschen Grosseltern

und

Madame Claudine Franquard,
Professeur de Physique-Chimie, Lycée Masséna, Nice

There is a crack. A crack in everything. That's the way the light goes in.

Mein besonderer Dank gilt

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für die Möglichkeit, diese Dissertation in seiner Arbeitsgruppe auszuführen
und für die Unterstützung, die mir während dieser Arbeit gewährt wurde.

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1 Foreword

This Ph.D. thesis is presented in two parts corresponding to the study of the peptaibol *Zervamicin II-2*, and to the study of the peptaibols *Hypomurocin A1*, *A3* and *A5*.

The numbering of all molecules as well as the literature references are continuous through all parts of the dissertation. The references are listed at the end of this dissertation.

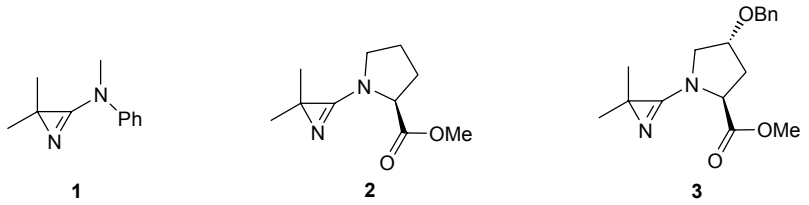
The experimental part of both parts is presented in a separate chapter at the end of this work.

An overview of the entire work is given in the following summary.

2 Zusammenfassung

Die Verwendung von 3-Amino-2*H*-azirinen in der Peptidsynthese für den Einbau von α,α-disubstituierte α-Aminosäuren ermöglicht die einfache Kettenverlängerung von Oligopeptiden bzw. Aminosäuren. Diese Einheiten (die Bekannteste davon ist Aib (**1**), 2-Aminoisobuttersäure oder 2-Methylalanin) kommen in der Natur selten vor und führen in Peptiden zu sterischen Hinderungen und eingeschränkten Konformationsfreiheiten.

Durch die Anwendung dieser Methode ('Azirin/Oxazolon Methode') wurden verschiedene Aib-enthaltende Peptaibole oder Segmente von Peptaibolen erfolgreich synthetisiert: das geschützte 6-16 Segment des *Zervamicins II-2* und die *Hypomurocine A1*, *A3* und *A5*. Die in den Synthesen eingesetzten 3-Amino-2*H*-azirine sind folgende (*Figur 1*):

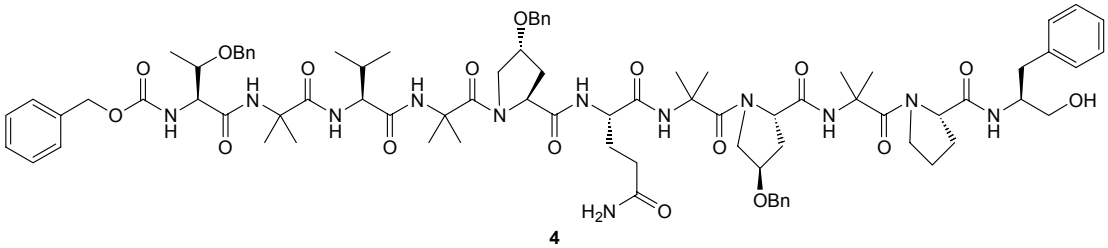


Figur 1. *3-Amino-2*H*-azirine* **1**, **2** *und* **3** *als Baueinheiten für Aib, Aib-Pro und Aib-Hyp*

Die Aib-Sequenz wird durch die Azirin-Kupplung mit **1** in eine Peptidkette eingebaut. Die Verwendung von **2** führt zum Einbau der Sequenz Aib-Pro und mit **3** wird die Sequenz Aib-Hyp eingebaut.

Der Vorteil der 'Azirin/Oxazolon-Methode' ist, dass es sehr einfach ist, diese disubstituierten α,α-Aminosäuren einzubauen, da keine Kupplungsreagenzien benötigt werden. Die Reaktion mit den 3-Amino-2*H*-azirinen **2** and **3**, die zwei Aminosäuren gleichzeitig einbauen, ist ebenfalls einfach und effizient. Durch die Verwendung dieser Dipeptid-Synthone wird die Kupplung zwischen einer Aib-Einheit und der ebenfalls sehr gehinderten Pro bzw. Hyp-Einheit vermieden.

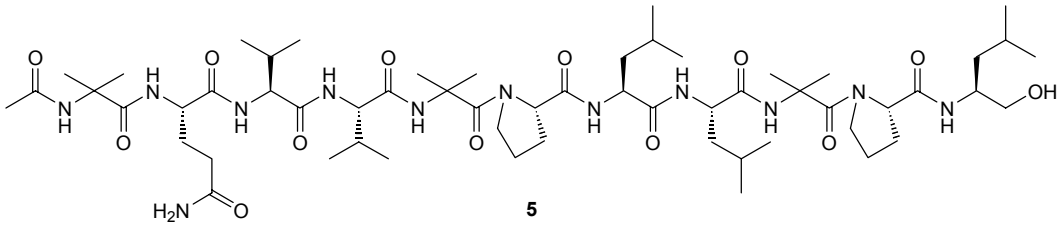
Der erste Teil dieser Dissertation befasst sich mit der Synthese des geschützten 6-16 Segments **4** des Peptaibols *Zervamicin II-2* (*Figur 2*), die mit Hilfe der 3-Amino-2*H*-azirine **1**, **2** und **3** mit guten bis sehr guten Ausbeuten gelang. Alle Versuche, Kristalle von **4** für die Röntgen-Kristallstrukturbestimmung zu erhalten, waren erfolglos.



Z-Thr(OBn)-Aib-Val-Aib-Hyp(OBn)-Gln-Aib-Hyp(OBn)-Aib-Pro-Pheol

Figur 2. *Geschütztes 6-16 Segment* **4** *des* Zervamicins II-2

Der zweite Teil behandelt die Totalsynthesen und Konformationsstudien der drei *Hypomurocine A1*, *A3* und *A5* (**5**, **6** und **7**; *Figur 3*). Die Synthesen gelangen unter der Verwendung des 3-Amino-2*H*-azirins **2** mit sehr guten Ausbeuten. Kristalle von verschiedenen kleinen und grossen Segmenten und von *Hypomurocin A3* (**6**), geeignet für die Röntgen-Kristallstrukturbestimmungen, wurden für alle drei *Hypomurocine* erhalten. Die Konformationstudien lieferten die, bei Anwesenheit von Aib, übliche Helix-Konformation, auch wenn die Peptide die Helix-brechende Aminosäure Pro enthält.



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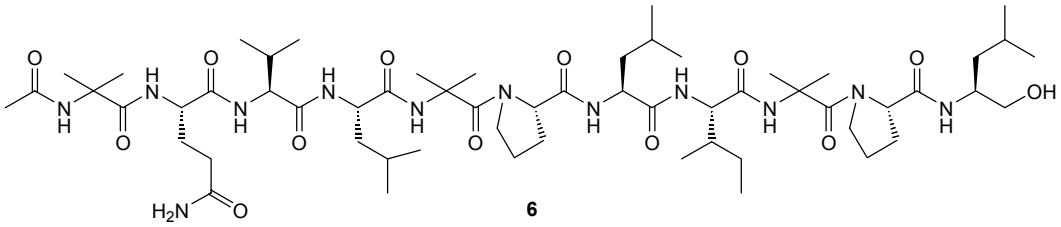
Ac-Aib-Gln-Val-Val-Aib-Pro-Leu-Leu-Aib-Pro-Leuol

Hypomurocin A1



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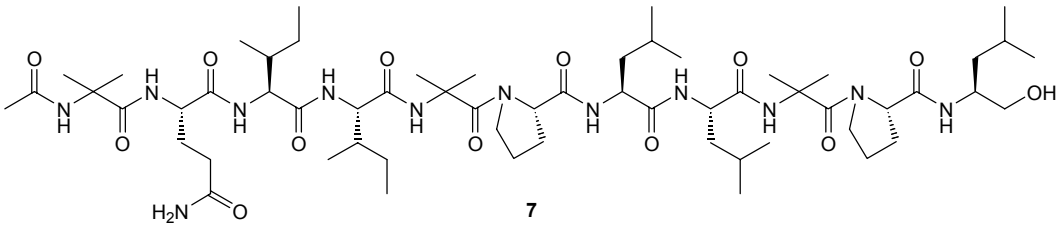
Ac-Aib-Gln-Val-Leu-Aib-Pro-Leu-Ile-Aib-Pro-Leuol

Hypomurocin A3



7

Ac-Aib-Gln-Ile-Ile-Aib-Pro-Leu-Leu-Aib-Pro-Leuol

Hypomurocin A5

Figur 3. *Die Undecapeptaibole* Hypomurocin A1, A3 *und* A5

Alle behandelten Projekte wurden mit Hilfe der ‘Azirin/Oxazolon-Methode’ erfolgreich und mit guten Ausbeuten abgeschlossen.

Zum Nachweis der Eigenschaft von Peptaibolen Ionenkanäle durch Membranen zu bilden, wurde eine Reihe von biologischen Testen mit Bakterien, menschlichen Zellen und Viren durchgeführt. Die Aktivität der Peptaibole wurde bestätigt, war jedoch schwach.

3 Summary

The use of 2*H*-azirin-3-amines in peptide synthesis for the introduction of α,α-disubstituted α-amino acids allows the easy chain elongation of oligopeptides and/or amino acids. In nature, these amino acids (the most famous of them being Aib (**1**), 2-aminoisobutyric acid or 2-methylalanine) are rarely present and peptides containing Aib are sterically hindered with limited conformational freedom.

By using 2*H*-azirin-3-amines, several Aib containing peptaibols or segments of peptaibols were successfully synthesized: the protected 6-16 segment of the *Zervamicin II-2* and the *Hypomurocins A1*, *A3* and *A5*. The involved 2*H*-azirin-3-amines in these synthesis are shown in *Figure 1*:

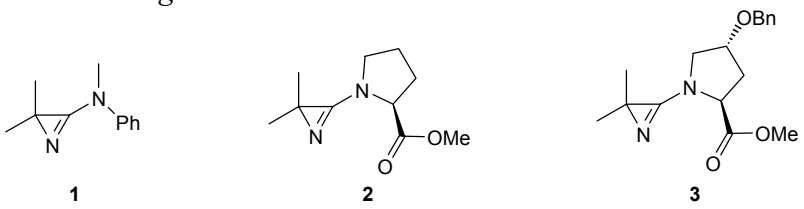
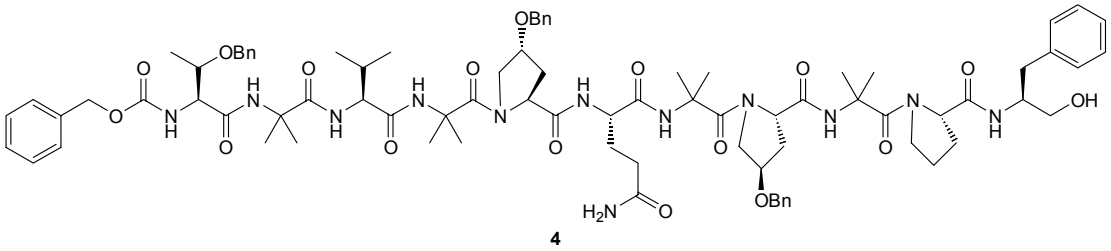


Figure 1. *2*H-*Azirin-3-amines* **1**, **2** *and* **3** *as Building Blocks for Aib, Aib-Pro and Aib-Hyp, respectively*

2*H*-Azirin-3-amine **1** is used to introduce Aib into a peptide chain. The dipeptide synthon **2** allows the introduction of the sequence Aib-Pro and with **3** the sequence Aib-Hyp is added.

The advantage of the 'azirine/oxazolone method' is the efficiency of the introduction of these highly hindered disubstituted α,α-amino acids without the need of coupling reagents. The use of the dipeptide synthons **2** and **3** is easy and efficient, and allows to avoid the difficult coupling between an Aib unit and the also hindered Pro or Hyp unit.

The first part of this dissertation deals with the synthesis of the protected 6-16 segment **4** of the peptaibol *Zervamicin II-2* (*Figure 2*), which was achieved in good to very good yields using the 2*H*-azirin-3-amines **1**, **2** and **3**. Attempts to get crystals suitable for the X-ray crystal-structure determination of **4** failed.

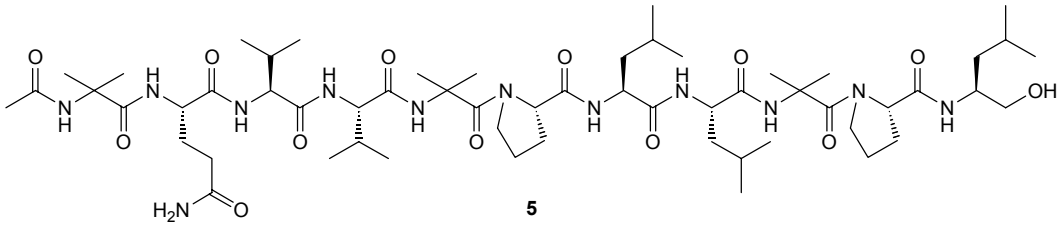


Z-Thr(OBn)-Aib-Val-Aib-Hyp(OBn)-Gln-Aib-Hyp(OBn)-Aib-Pro-Pheol

Figure 2. *Protected 6-16 Segment* **4** *of* Zervamicin II-2

The second part concerns the total synthesis and conformational studies of the three *Hypomurocins* A1, A3 and A5 (**5**, **6** and **7**; *Figure 3*). The syntheses were

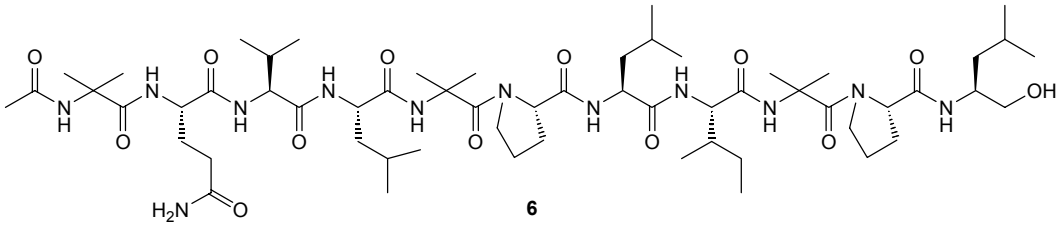
performed by using the 2*H*-azirin-3-amine **2** with very good yields. Crystals of various small and long segments and *Hypomurocin A3* (**6**), suitable for the X-ray structure analysis, could be obtained in each series. The conformational studies have shown the expected helical conformation due to the presence of Aib, even if it is immediately followed by the 'helix breaker' unit Pro.



5

Ac-Aib-Gln-Val-Val-Aib-Pro-Leu-Leu-Aib-Pro-Leuol

Hypomurocin A1



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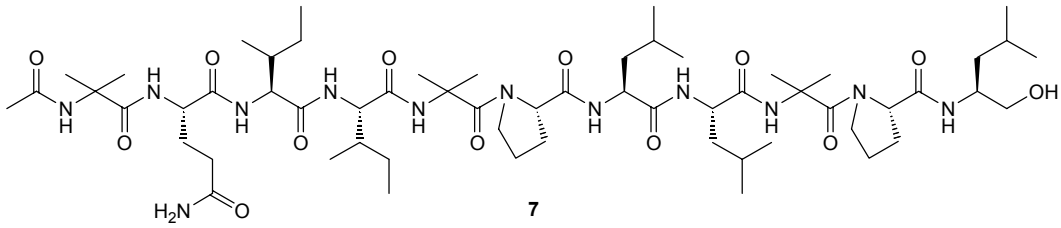
Ac-Aib-Gln-Val-Leu-Aib-Pro-Leu-Ile-Aib-Pro-Leuol

Hypomurocin A3



7

Ac-Aib-Gln-Ile-Ile-Aib-Pro-Leu-Leu-Aib-Pro-Leuol

Hypomurocin A5

Figure 3. *The Undecapeptaibols* Hypomurocins A1*,* A3 *and* A5 *resp.* **5***,* **6** *and* **7**

In conclusion, all projects were successfully achieved with good yields by using the 'azirine/oxazolone method'.

In addition, to test the property of peptaibols to form ion channels through membranes, a series of biological tests with bacteria, human cells and viruses was carried out. The activity of the peptaibols was confirmed, although it was weak.

4 Topic of the Present Dissertation

All syntheses in this dissertation are solution phase syntheses.

4.1 *Zervamicin II-2*

The protected 6-16 segment **4** of the *Zervamicin II-2*, which, in its unprotected form, is also common to *Zervamicin II-5*, was selected as a model for the demonstration of the use of the azirine/oxazolone method in peptaibol synthesis [1, 2]. The aim of the present synthesis is to show that the introduction of the residues Aib, Aib-Pro, and Aib-Hyp in the selected segment **4** can be carried out in an elegant way by using this method.

4.2 *Hypomurocins A1*, *A3* and *A5*

As a second topic, the *Hypomurocins* were chosen because they contain two Aib-Pro sequences. Moreover, due to the high similarity of the molecules of the *Hypomurocin A* family and to make a structural comparison by three-dimensional characterization, it was decided to synthesize three *Hypomurocins* of the A family. The *Hypomurocins A1*, *A3* and *A5* were selected arbitrarily.

Additional work to the original project was the comparison of the synthetic *Hypomurocins A1*, *A3* and *A5* with a sample of the natural *Hypomurocin* A family. This comparison was successfully achieved by HPLC-MS analysis.

Finally, the biological activity and the toxicity of the three synthetic *Hypomurocins A1*, *A3* and *A5* (**5**, **6** and **7**) were tested by running *in-vitro* tests with various biological material like bacteria, blood Agar plates, viruses and human cells.

5 Introduction

5.1 Peptaibols

In the coming decades, the synthesis of large quantities of peptides (from more than 100 kg up to a multi-tonne scale per year) will be a great challenge. In the 1990's it was generally thought that large-scale syntheses of peptides (up to 50 kg/year) are economically nonsensical because of the inadequate production technology and the low demand of peptides as pharmaceuticals. Simply stated, it was difficult and expensive to synthesize peptides of more than 30 amino acids [3]. Since the knowledge of the synthesis of peptides has increased as well as the interest in the treatment of diseases by using peptides, it is to be expected that this field will be soon become industrially more developed, thereby allowing access to low-cost material.

Since the first solid-phase peptide synthesis (SPPS) by *Merrifield* [4] in 1963, interest in peptide synthesis has grown significantly, and a convenient way to prepare long peptides was developed quite quickly. Nowadays, these syntheses are often fully automated. Although this technique is still very common, it has some limitations on purification and yields depending on the length of the peptide, and the batch is limited to a few dozen kilograms per reactor. For larger batches, solution phase synthesis (SPS) has proven to be a superior method. Moreover, whereas SPPS is a linear synthesis, SPS allows a convergent preparation in which the amount of starting materials and, therefore, the production costs can be reduced. For instance, expensive amino acids can be introduced at a later step of the synthesis. Based on this and other optimization factors, optimization matrices such as *Hadamard* matrices [5], have been developed and have allowed to evaluate the fastest and cheapest synthesis.

Peptaibols are membrane-active oligopeptides isolated from fungal sources. They have an antibacterial property due to their self-association in lipid membranes forming ion channels through membranes [6]. They are linear amphiphilic oligo**pept**ides containing 11 to 20 amino acids characterized by the content of up to 50% of the non-proteinogen amino acid **Aib** (α-aminoisobutyric acid) and the presence of an acetylated *N*-terminus and a reduced *C*-terminus alcoh**ol** function (*e.g.* Valol, Leuol, Pheol) [7, 8], standing for the name 'pept-aib-ol' [9]. They also often contain the Iva (α-ethyl alanine) residue [10, 11]. Aib is the simplest α,α-disubstituted amino acid present in nature [12], and it is well known for its ability to induce helical folding of the peptide chain [13]. This effect still dominates when amino acids such as proline (Pro) and hydroxyproline (Hyp), known as “helix breaker”, are present [14].

Moreover, in a series of articles [15-26], the extraterrestrial origin of Aib and Iva found in comet dust in the *Cretaceous/Tertiary* boundary has been debated. The origin was confirmed, *e.g.* with the *Murchison* meteorite, which fell in Australia in 1969 and was the first proof of amino acids in extraterrestrial material.

Recent work [27-30] on various peptaibols, *i.e. Alamethicin F-30* (Akaji and Kiso, 1995) [31], *Zervamicin II-B* (Ogrel and Raap, 1997) [32], *Trichovirin I* (Brückner and Kosa, 2003 [33]; Luykx and Heimgartner, 2003 [34]), the crystal structure of *Antiamoebin* (Karle and Balaram, 1998) [35], *Peptaibolin* (Crisma and Toniolo, 2001) [36], lipopeptaibols (Toniolo, 2001) [37], the octameric model for the *Trichotoxin* channel (Chugh and Wallace, 2002) [38, 39], illustrating their characterisation,

structural study as well as their total synthesis, or simply the discovery of new peptaibols like *Cervinin* from Mycogone cervina (Wilhelm and Sterner, 2004) [40], confirm the interest and the growing accessibility of this chemistry. The first online peptaibol database [41], recording over 300 molecules, and being developed and maintained in London/UK by *Chugh* and *Wallace* is now accessible. It illustrates the need for 'peptaibolists', *i.e.* the community of chemists working on peptaibols, to have an updated and accurate system with the latest developments on peptaibols from the name, sequences and fungal origin to the known X-ray structures and three-dimensional NMR studies.

The antibiotic property of peptaibols is assumed to be a consequence of the helix conformation of these Aib containing molecules. The peptaibol enters the membrane of the cell and by self association of several molecules to form *n*-mers, *n* going up to 14 but is assumed to be in general 8. The *n*-mer creates an ion channel, leading to the destabilization of the cell and finally to its dead or to the inhibition of its function (*Figure 4*) [38], although the characterization of the *n* value has to be carefully subjected to the models [39].

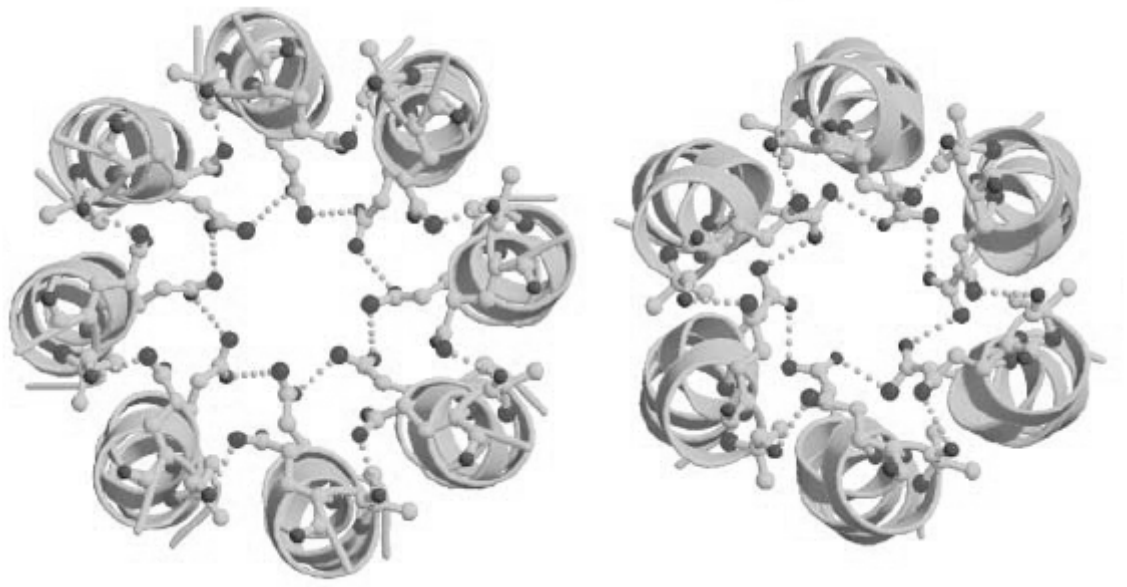


Figure 4. *Octameric and Hexameric Models for* Trichotoxin *Channels* [38, 39]

Moreover, if the length of the peptaibols is smaller than the width of the lipid membrane, the peptaibols are also assumed to self associate in a head-to-tail [30] or both head-to-tail and/or head-to-head fashion [42] (*Figure 5*). In the case of the head-to-head association, the polarity barrier due to the inversion of the second peptaibol chain in the channel has not been explained, and this model might not be appropriate with regard to the diffusion of the ions.

The introduction of Aib into peptaibols was a difficult synthetic task for long time (see [33]). During the last ten years, these difficulties have been surmounted by the development of highly reactive coupling reagents and activated amino acids [32, 43, 44]. Furthermore, the fluoride activation of α,α-dialkylated α-amino acids and peptides has been shown to allow convenient peptaibol synthesis on the solid phase [45, 46]. Although the solid phase peptide synthesis is now the method of choice for routine, general and automated preparation of peptaibols, the solution phase synthesis has also

some advantages. For example, any defined segment, not only the final product, can be obtained, *e.g.* for structure analysis by X-ray crystallography and for testing the bio-activity.

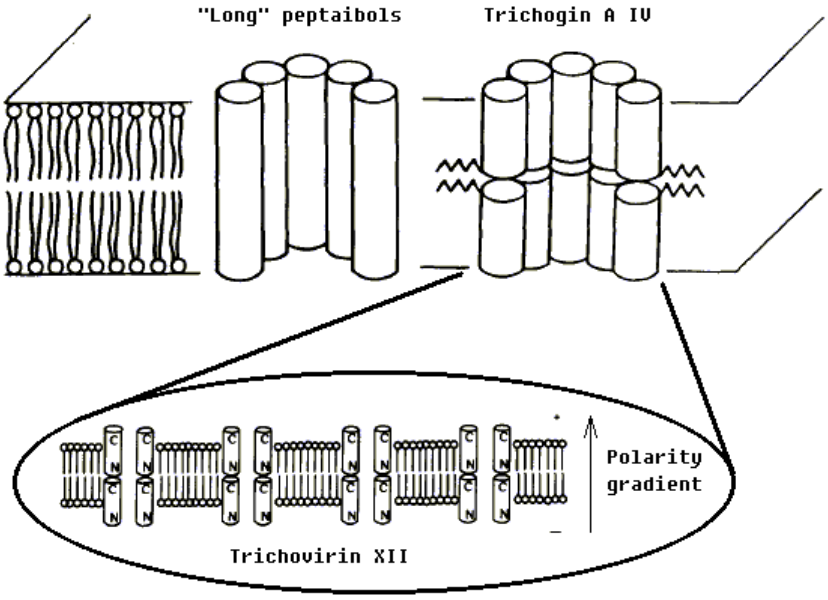


Figure 5. *Postulated Head-to-Tail Dimerization Models for Short Peptaibols (cf.* [30]*)*

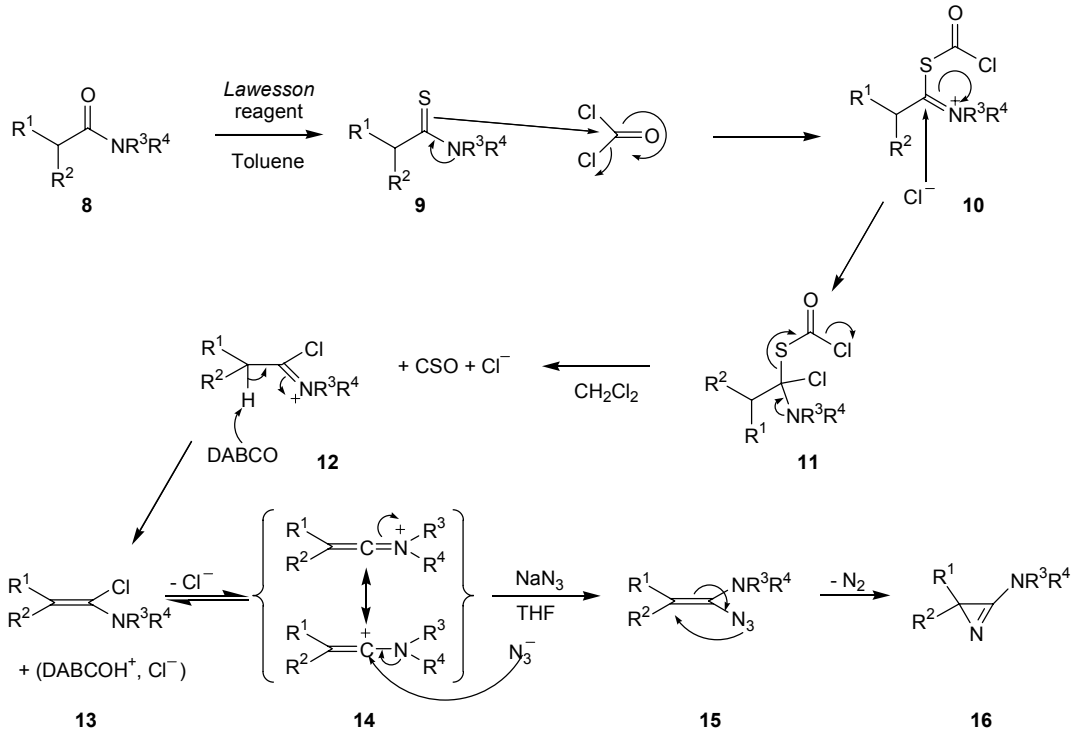
5.2 2*H*-Azirin-3-amines

2*H*-Azirin-3-amines are three-membered amidines with a C-N double-bond in endocyclic position. The most general synthesis is based on the procedure of *Rens* and *Ghosez* [47]. A method, which avoids the use of phosgene, has been developed by *Villalgordo* and *Heimgartner* [48] leading from the starting amide via an enolate intermediate to the chloroenamine and the azirine.

The synthetic possibilities to use 2*H*-azirin-3-amines in organic chemistry are numerous [2], and in the field of peptide chemistry, they are of particular help for the construction of linear peptides with α,α-disubstituted α-amino acids, taking into account that until the end of the 1990's, conventional chain elongation techniques either failed or suffered from extreme difficulties. The general synthetic way to obtain 2*H*-azirin-3-amines is presented in *Scheme 1*.

The use of thioamides, prepared by treatment with *Lawesson* reagent [49, 50], does not belong to the original route described by *Rens* and *Ghosez*, but it allows to considerably increase the yield of **16** [51]. The formation of the α-chloroenamine **13** occurs *via* nucleophilic attack of the thioamide **9** on the highly reactive phosgene. By treatment of the ketiminium salt **14** with natrium azide, **15** leads to the formation of the three-membered ring **16** with irreversible loss of nitrogen.

*Scheme 1. Synthesis of 2*H-*Azirin-3-amines*



5.3 The Azirine/Oxazolone Method

Regarding the synthesis of peptaibols, at the beginning of the 90's *Heimgartner* developed a synthetic method to introduce in solution highly hindered α,α-disubstituted α-amino acids like Aib and Iva into peptides *via* the azirine/oxazolone method [2]. More recently *Luykx* [51], *Pradeille* [52] and *Breitenmoser* [53] have illustrated the advantage of this method by the introduction of amino acids like Aib or dipeptide units like Aib-Pro and Aib-Hyp by using the corresponding 2*H*-azirin-3-amines **1**, **2** and **3**, respectively, as building blocks in peptide chain elongations, where conventional techniques failed or gave low yields.

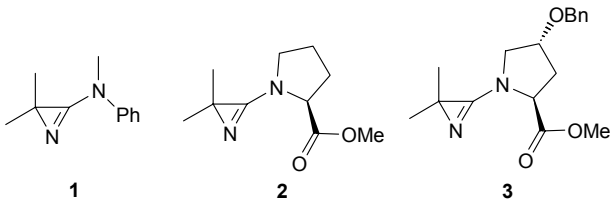


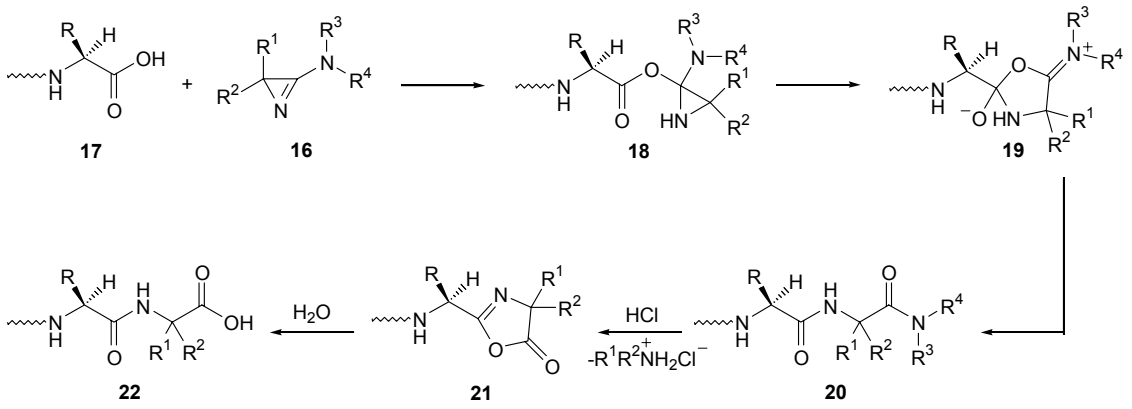
Figure 6. *2*H-*Azirin-3-amines as Building Blocks for Aib, Aib-Pro and Aib-Hyp*

Until a few years ago, the introduction of such sterically demanding amino acids was a problem, because the formerly predominant coupling techniques with carbodiimides or *via* active esters did not allow these reactions to be performed with reasonable yields [54-60]. Nowadays, these methods have been replaced by those which

utilize new coupling reagents and activating additives, such as uronium and phosphonium salts, which permit to carry out these coupling reactions with good yields and without any appreciable epimerization or side product formation [61-70].

The reaction mechanism of the coupling of an amino or peptide acid **17** with 2*H*-azirin-3-amines **16** is shown in *Scheme 2*. Protonation of N(1) of the azirine followed by nucleophilic addition of the carboxylate leads to aziridine intermediate **18**, which undergoes a ring enlargement to give zwitterion **19**. Ring opening of the latter then yields peptide **20**. Hydrolysis of **20** under mild conditions (3N HCl, THF/H_2O, r.t.) gives the peptide acid **22**. It has been shown that oxazolone **21** is an intermediate and can be prepared from **20** by treatment with gaseous HCl in non-nucleophilic solvents in high yield. The direct coupling of **21** with an amino component is also possible [2, 71].

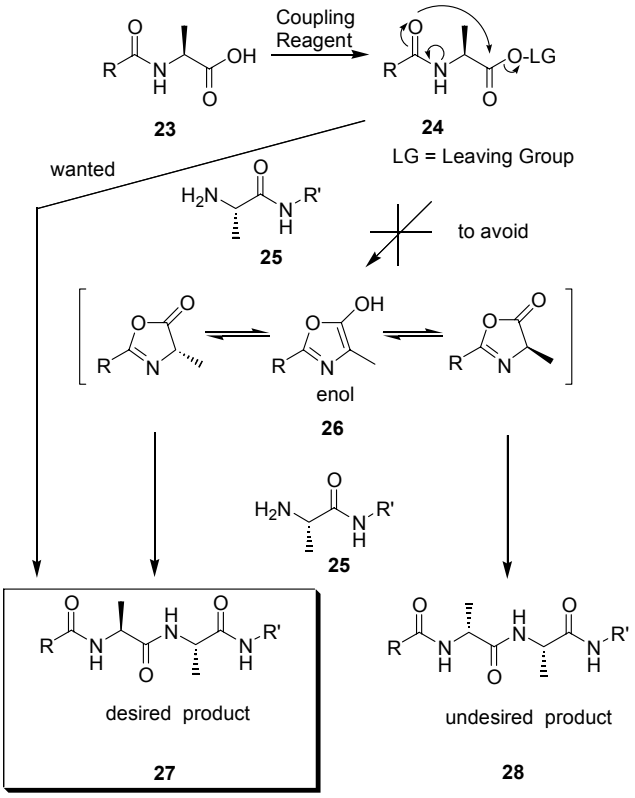
*Scheme 2. Reaction Mechanism of the Coupling of an Amino or Peptide Acid with 2*H*-Azirin-3-amines*



5.4 Coupling Reagents and Additives

For the coupling of two amino acids, they have to be activated with the help of coupling reagents. Moreover, there is also the risk of epimerization during the coupling (*Scheme 3*). In order to lower this risk, additives such as aminium/uronium and phosphonium salts like HOAt and HOBt are added to the reaction mixture [70, 72]. The currently accepted mechanism in the process of the racemization is based on an oxazolone intermediate **26**, from which *via* enolization both epimers can be formed. It has been widely proved that the addition of HOBt as an additive was very efficient to lower this racemization [73].

Scheme 3. Mechanism of the Epimerization during the Coupling



5.5 The Ramachandran Plot

The structure of peptides and proteins are classified in primary, secondary, tertiary and quaternary structures, and the properties of these molecules are strongly linked to their three-dimensional arrangement.

The primary structure corresponds to the sequence of amino acids in the peptide chain. This chain, which contains the amide bonds, is called the peptide backbone.

The secondary structure of a segment of a polypeptide chain is the local spatial arrangement of its main-chain atoms without regard to the conformation of its side chains or to its relationship with other segments (IUPAC-IUB, 1970). The secondary structure is characterized by the geometry of these chains and is defined by the ω_i amide bond torsion angle and two other torsion angles: φ_i being the dihedral angle about the C_i-N bond and ψ_i being the dihedral angle about the C_i-CO bond for a given α-C_i atom (*Figure 7*). Due to the delocalization of the lone-pair of the N-atom and the π-electrons of the amide bond, the amide bond is almost planar and, therfore, the three-dimensional arrangement is a result of φ_i and ψ_i.

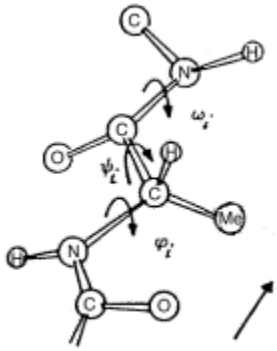


Figure 7. *Torsion Angles* ω_i, φ_i *and* ψ_i *in a Peptide Chain*

The two torsion angles φ_i and ψ_i may take all possible values, but actually due to energy reasons, only certain values are allowed. The graphic visualization of these values is commonly used to determine the type of secondary structures taken by the parts of the peptide chain and is called the *Ramachandran* Plot [74]. The conformational freedom for a given amino acid in a peptide chain is illustrated in *Figure 8*. If we compare Ala (alanine) and Aib (methyl alanine), we immediately see the much more restricted conformation of Aib due to the presence of a second methyl group as a side chain at the α-C atom.

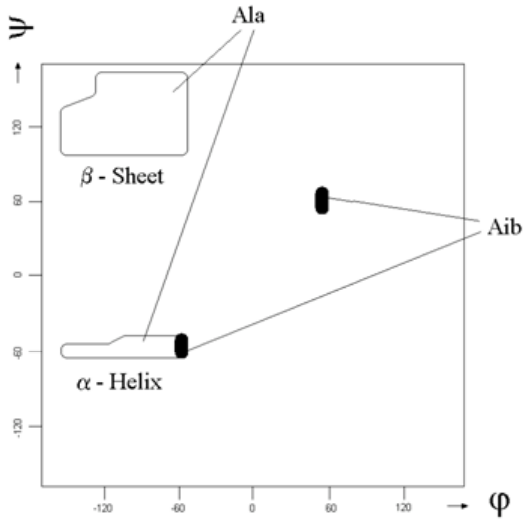
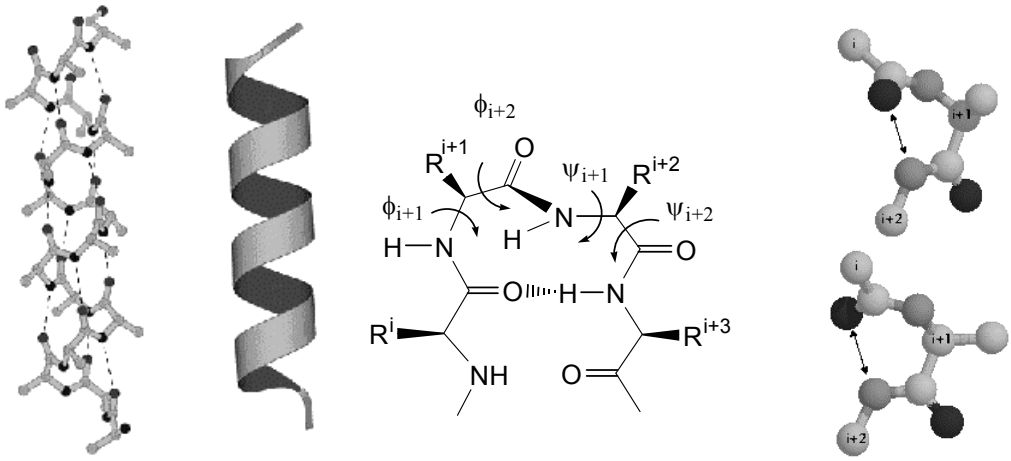


Figure 8. *The* Ramachandran *Plot for Ala and Aib*

There are mainly three types of regular structures in a peptide chain (helix, turn and inverse turn) according to the φ_i and ψ_i values (*Figure 9*).



α - Helix β - Turn γ - and inverse γ - Turn

Figure 9. α-*Helix,* β-*Turn and* γ-*Turn: the Three General Types of Secondary Structures in a Peptide Chain*

In the helix, the amino acid chain is twisted around a central axis in a regular manner and the structure is stabilized by intrachain C=O----H-N H-bonds, the side chains are pointing out of the helix. There are different types of helices (α_L, α_R, 3_{10}, π, poly(Pro) I and II, and poly(Gly) II) distinguished by the H-bonding pattern, number of residues per turn and the tightness of the coil. The classical (most common) right handed α_R-helix has an i+4 H-bonding pattern.

The idealized torsion angles φ_i and ψ_i for some secondary structures of peptides are given in *Table 1*.

Table 1. *Torsion Angles for some Secondary Structures of Peptides*

Conformation	φ_{i+1} (°)	ψ_{i+1} (°)	φ_{i+2} (°)	ψ_{i+2} (°)	n	A	H-bond
α-helix (right handed)	-57	-47	-57	-47	3.6	13	i,i+4
3_{10}-helix	-74	-4	-74	-4	3.0	10	i,i+3
π-helix	-57	-70	-57	-70	4.4	16	i,i+5
Parallel β-sheet	-119	+113	-119	+113	2.0		
Antiparallel β-sheet	-139	+135	-139	+135	2.0		
β-Turn type I and I'	±60	±30	±90	0		10	
β-Turn type II and II'	±30	±120	±80	0		10	
β-Turn type III and III'	±60	±30	±60	±30		10	
γ-Turn	+70 to +85	-60 to -70				7	
Inverse γ-Turn	-70 to -85	+60 to +70				7	

n: number of residues per helical turn; A: atoms in the H-bonded loop.

The tertiary structure is the three-dimensional arrangement of the whole polypeptide. This includes the arrangement of the helices, β-sheets and turns as well as eventually existing sulfur-sulfur bridges.

Some proteins consist of an assemblage of several tertiary structures, which is called the quaternary structure. This is the case for instance with the four independent protein chains of Hemoglobin which become an oligomer protein through self assemblage.

6 The Protected 6-16 Segment of *Zervamicin II-2*

6.1 Introduction

The 16-residue peptaibols of the *Zervamicin* family are produced by *Emericellopsis Salmosynnemata*. In 1981, *Rinehart et al.* have reported the structure of eleven *Zervamicin* structures [75], and the crystal structure of Leu[1]-*Zervamicin II B* has been established by *Karle et al.* in 1991 [76-78]. The solution phase synthesis of *Zervamicin II B* was performed by *Ogrel et al.* [32].

The selected highlighted protected 6-16 segment **4** is common to *Zervamicin II-2* and *Zervamicin II-5* in its unprotected form (*Figure 10*):

Z-Thr(OBn)-Aib-Val-Aib-Hyp(OBn)-Gln-Aib-Hyp(OBn)-Aib-Pro-Pheol
4

Ac-Trp-Ile-Gln-Aib-Ile-**Thr-Aib-Val-Aib-Hyp-Gln-Aib-Hyp-Aib-Pro-Pheol**
Zervamicin II-2

Ac-Trp-Ile-Gln-Iva-Ile-**Thr-Aib-Val-Aib-Hyp-Gln-Aib-Hyp-Aib-Pro-Pheol**
Zervamicin II-5

Figure 10. Zervamicins II-2 *and* II-5

In the past few years, the azirine/oxazolone method has been shown to provide an efficient and elegant way to extend peptide chains with Aib, Aib-Pro and Aib-Hyp units by using the 2*H*-azirin-3-amine derivatives **1**-**3** as building blocks. The preparation of these synthons has been described recently (**1** [79], **2** [51], **3** [53]). By using this methodology, segments of the peptaibols *Alamethicin F-30* [1], *Trichotoxin A-50(G)* [80, 81], *Antiamoebin I* [81-83], and *Trichovirin I 1B* [51, 84] were prepared. Recently, the conventional solution phase synthesis of *Trichovirin I 4A* was described [33]. The successful coupling reactions with **1** and **2** have already been reported [1, 2, 51]. Their coupling potential is now illustrated in the reactions with Z-Thr(OBn)-OH (**29**) (*Scheme 7*) and Z-Gln-Aib-Hyp(OBn)-OH (**30**) (*Scheme 8*), respectively. Furthermore, the reactions of **3** with Z-Val-OH and Z-Gln-OH show its potential as an Aib-Hyp synthon.

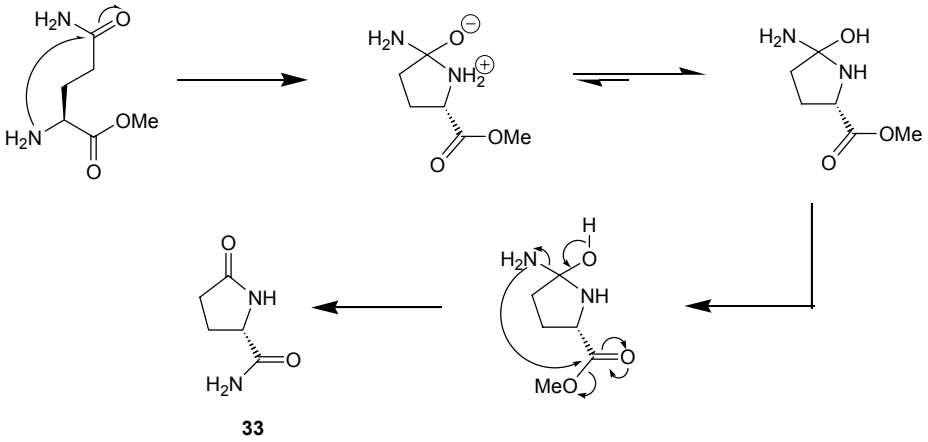
6.2 Synthesis

6.2.1 Strategy of the Synthesis

In the protected 6-16 segment of *Zervamicin* II-2, there are three different Aib or dipeptide units which can be introduced *via* the azirine/oxazolone method by using the building blocks **1**, **2**, and **3**, and which makes this molecule a versatile target for our chemistry. The first idea was to split the molecule in two equal parts. Taking into consideration the position of the different Aib units, the strategy was to prepare the molecule **4** by condensation of a penta- and an hexapeptide, *i.e.* from Z-Thr(OBn)-Aib-Val-Aib-Hyp(OBn)-OH (**31**) and H-Gln-Aib-Hyp(OBn)-Aib-Pro-Pheol (**32**; see *Scheme 6*). Moreover, if a strategy involves the use of H-Gln-OMe (for instance to be coupled

with **31**), caution must be taken because this compound rearranges easily to give **33** (within 2-3 days). A proposed reaction mechanism is shown in *Scheme 4*. This side reaction might be the reason, why H-Gln-OMe is commercially not available.

Scheme 4. Proposed Reaction Mechanism for the Formation of **33**



After deprotection of the amine group, an intramolecular nucleophilic attack of the nitrogen electron pair leads to the formation of the heterocycle **33**.

Suitable crystals of **33** for an X-ray crystal-structure determination were obtained (*Figure 11*). The crystals are enantiomerically pure, however the absolute configuration of the molecule has not been determined. The enantiomer used in the refinement was based on the known *S*-configuration of the starting material. The structure of this molecule has previously been reported as the monohydrate [85]. Each amide group of the molecule acts as a donor for H-bonds. The cyclic amide group forms an intermolecular H-bond with the cyclic amide O-atom of a neighbouring molecule. This interaction links the molecules into infinite chains. One of the H-atoms of the exocyclic amide group forms an intermolecular H-bond with the cyclic amide O-atom of a different neighbouring molecule and yields infinite chains. The other H-atom of the exocyclic amide group forms an intermolecular H-bond with the exocyclic amide O-atom of a third neighbouring molecule and yields infinite chains. The combination of all interactions links the molecules into an infinite three-dimensional framework.

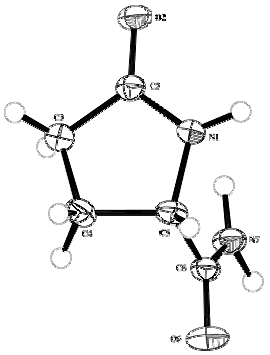
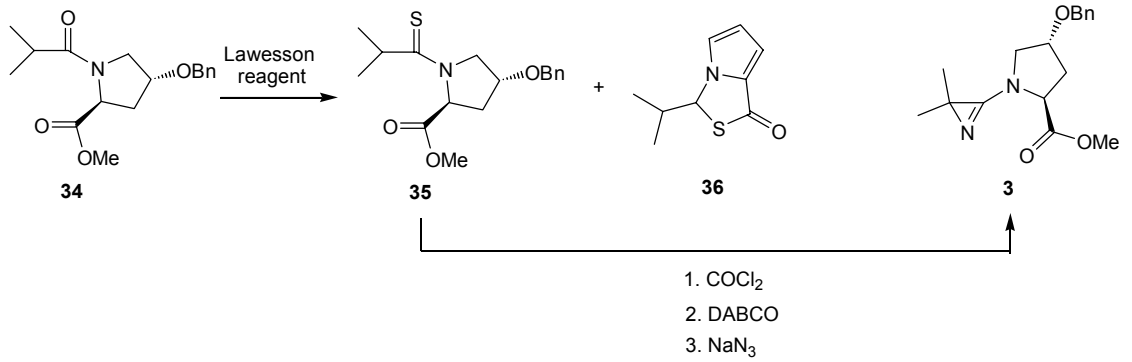


Figure 11. *ORTEP Plot* [86] *of the Molecular Structure of* **33** (50% probability ellipsoids; arbitrary numbering of the atoms)

6.2.2 Preparation of the 2*H*-Azirin-3-amine(s)

The preparation of the 2*H*-azirin-3-amines **1**, **2** and **3** actually concerned only that of **3** as the two others were already available in the laboratory. According to *Breitenmoser's* procedures [53], 1.63 g of **3** were synthesized from the thioamide **35**and purified by at least two successive flash column chromatographies. Although the reaction time of the treatment of the corresponding amide **34** with *Lawesson* reagent did not exceed 25 min, the side product **36** formed by the initial cyclization and subsequent elimination of BnOH as reported in [53] was also observed here (*Scheme 5*). The yield of this product was not determined.

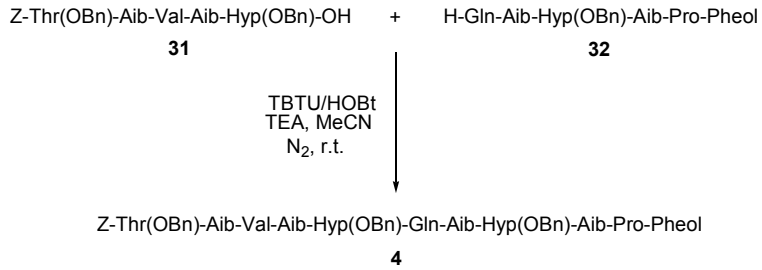
*Scheme 5. Preparation of the 2*H*-Azirin-3-amine* **3**



6.2.3 Unsuccessful Synthesis

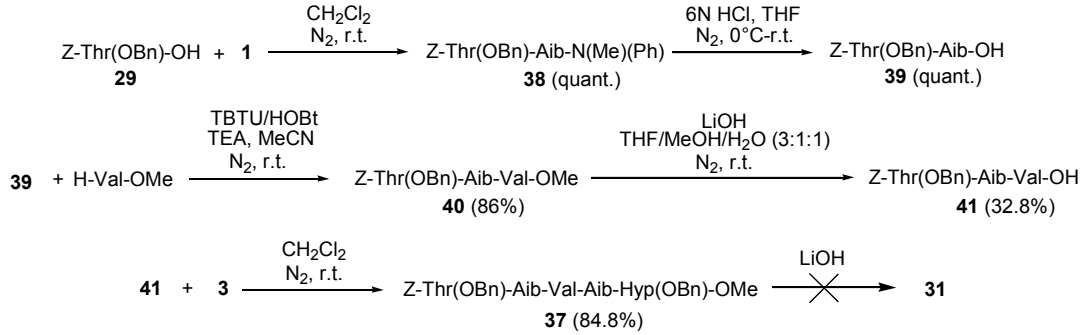
The first strategy was to prepare the protected segment **4** by the coupling of the pentapeptide **31** with the C-terminal hexapeptide **32** (*Scheme 6*).

Scheme 6. First Strategy for the Preparation of **4**



The synthesis of the protected ester **37**, which is the precursor of the desired segment **31**, was achieved according to *Scheme 7*: coupling of **29** with azirine **1** followed by selective hydrolysis gave **39** in quantitative yield. Coupling of the latter with H-Val-OMe by using TBTU/HOBt yielded the tripeptide **40**. Unexpectedly, saponification of the ester with LiOH gave the acid **41** in only 32.8% yield. Reaction with the Aib-Hyp synthon **3** led to **37** in 84.8% yield.

Scheme 7. Synthesis of the Protected Ester **37**



Unfortunately, the planned synthesis of **31** according to *Scheme 6* could not be achieved, because the hydrolysis of its methyl ester Z-Thr(OBn)-Aib-Val-Aib-Hyp(OBn)-OMe (**37**) failed. This surprising result was confirmed by several attempts to hydrolyse **37** under different conditions. According to ^{1}H-NMR and MS analysis, the molecule was destroyed, with lost of the Z protecting group as well as the benzyloxy group. The reason of this unexpected reaction remains unknown. Thus, the coupling between **31** and **32** could not be tried, and the whole strategy had to be revised.

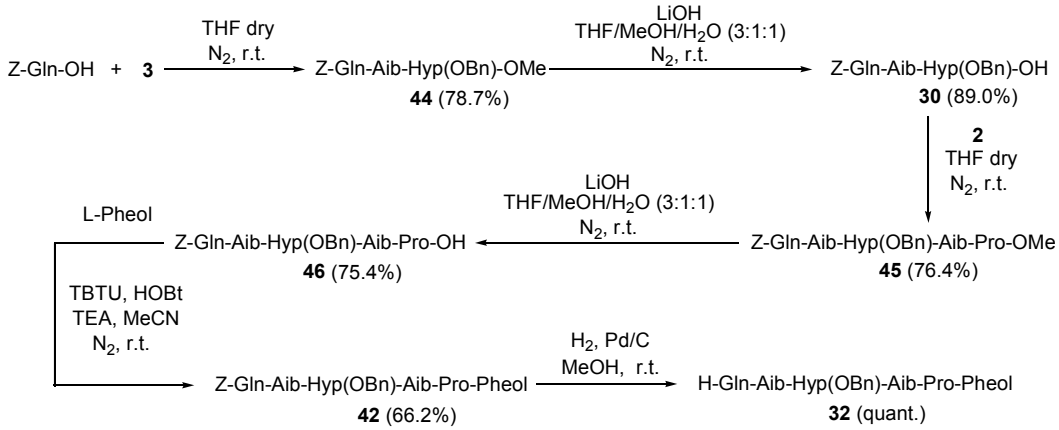
It is interesting to notice that the only hydrolysis in this synthesis of **37** (**40**→**41**) yielded the expected product, but in very low yield, indicating a possible influence of the threonine part of the molecule on the hydrolysis. Furthermore, the purification of **41** after the work up was difficult.

6.2.4 Successful Synthesis

As a consequence of the failure of the first strategy, we decided to synthesize **4** from the three sub-segments Z-Gln-Aib-Hyp(OBn)-Aib-Pro-Pheol **42**, Z-Val-Aib-Hyp(OBn)-OH **43**, and Z-Thr(OBn)-Aib-OH **39**. These three segments should then be coupled by using the well-known TBTU/HOBt method that should avoid epimerisation during segment condensations. Indeed, no epimerisation could be detected by NMR analysis. The protective groups of the amino groups and the side chain hydroxy groups were chosen to be stable under acidic and basic conditions [87]. Therefore, Z-protected amino acids were used, and the OH group of Thr was benzylated as the OH group of the Aib-Hyp synthon was.

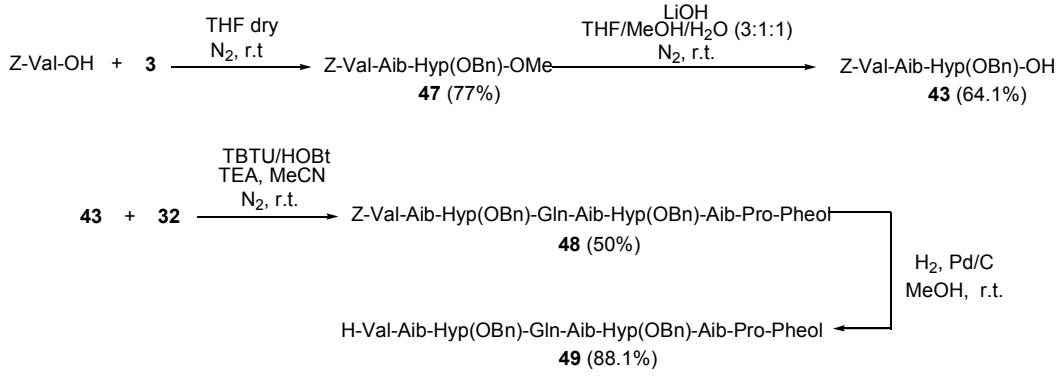
The reaction of the Z-protected glutamine residue (Z-Gln-OH) with the Aib-Hyp dipeptide synthon **3** in THF at room temperature gave the tripeptide **44** in 79% yield (*Scheme 8*). Basic hydrolysis of the ester group of the latter with LiOH in THF/methanol/water led to **30,** which was coupled with azirine **2**, the Aib-Pro dipeptide synthon, to yield the pentapeptide **45**. This azirine coupling was performed with 76% yield. Another basic hydrolysis gave the pentapeptide acid **46**, which was coupled with L-Pheol by using the standard TBTU/HOBt method to give the C-terminal hexapeptide **42** in 66.2% yield. After deprotection of the N-terminus by hydrogenolysis, the glutamine segment **32** was obtained in quantitative yield. It is worth mentioning that the benzyloxy protecting group of the Hyp residue was stable under the chosen conditions (10% Pd/C).

Scheme 8. Synthesis of the Hexapeptide **32**



Analogously, the reaction of the Z-protected valine residue Z-Val-OH with the Aib-Hyp dipeptide synthon **3** led to the tripeptide **47** in 77% yield (*Scheme 9*). The methyl ester group of the latter was hydrolyzed by treatment with LiOH in THF/methanol/water to give **43** in 64.1% yield. Using the TBTU/HOBt method, **43** was coupled with the glutamine 11-16 segment **32** to give the C-terminal nonapeptide **48** in 50% yield. Deprotection by hydrogenolysis of the N-terminus of the latter gave the 8-16 segment **49** in 88.1% yield.

Scheme 9. Synthesis of the Nonapeptide **49**



Crystals of **47**, suitable for an X-ray crystal-structure determination, could be obtained from a mixture of methanol, hexane and ethyl acetate (*Figure 12*). The space group permits the compound in the crystal to be enantiomerically pure, but the absolute configuration of the molecule has not been determined. The enantiomer used in the refinement was based on the known *S*-configuration of the Val moiety. Relative to the (*S*)-Val moiety, C(8) and C(26) have the expected *S*- and *R*-configuration, respectively. The amide groups form intermolecular hydrogen bonds which link the molecules into two-dimensional networks. The amide group of Val forms an intermolecular H-bond with the ester carbonyl O-atom at the opposite end of a neighboring molecule. This interaction links the molecules into extended chains. The Aib amide group forms an intermolecular H-bond with the adjacent amide O-atom of a different neighboring molecule. This interaction also links the molecules into extended chains. The combination of these interactions links the molecules into two-dimensional networks.

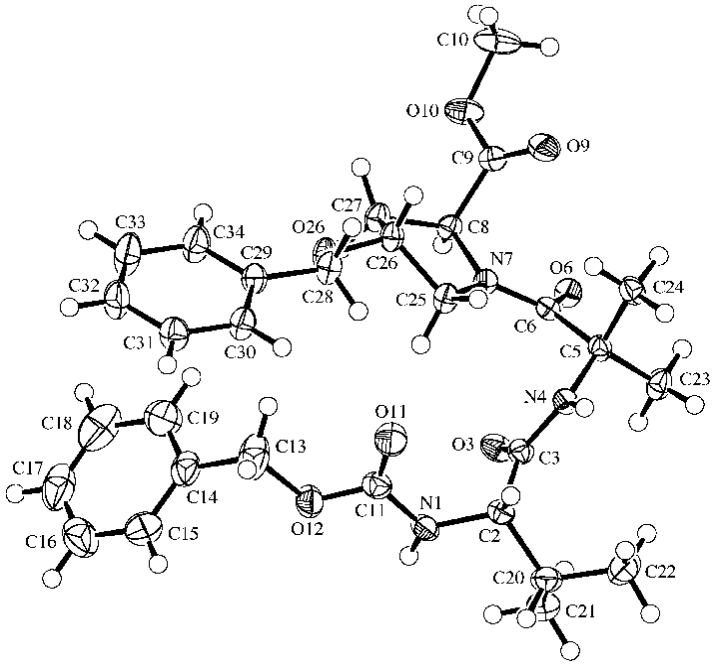
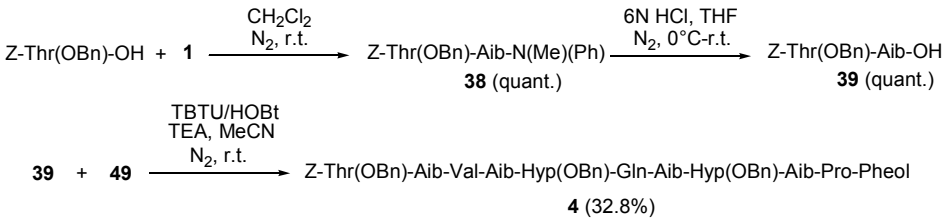


Figure 12. *ORTEP Plot* [86] *of the Molecular Structure of* **47** (50% probability ellipsoids; arbitrary numbering of the atoms)

The treatment of the OBn- and Z-protected threonine residue Z-Thr(OBn)-OH with the Aib synthon **1** in CH_2Cl_2 at room temperature gave the dipeptide **38** in quantitative yield (*Scheme 10*). This latter was hydrolyzed under the usual conditions, *i.e.* with 3N HCl in a 1:1 mixture of THF/water, and the dipeptide **39** acid was obtained in quantitative yield. The final coupling of **39** with the 8-16 segment **49** was achieved by using the TBTU/HOBt method, and the C-terminal undecapeptide **4** was obtained in 32.8% yield.

Scheme 10. Synthesis of the Undecapeptide **4**



Crystals of **38** suitable for an X-ray crystal-structure determination were obtained from $CDCl_3$ by slow evaporation of the solvent (*Figure 13*). The space group permits the compound in the crystal to be enantiomerically pure, but the absolute configuration of the molecule has not been determined. The enantiomer used in the refinement was based on the assumption that the molecule has the 11*S*-configuration. Relative to this, C(23) has the *R*-configuration. The molecule forms one intramolecular and one intermolecular hydrogen bond, with the latter linking the molecules into extended chains. The Aib amide group forms an intermolecular H-bond with the amide O-atom of the secondary amide group of a neighboring molecule. This interaction links

the molecules into extended chains. The amide group of Thr forms a weak intramolecular H-bond with the benzyloxy O-atom, thereby forming a five-membered loop.

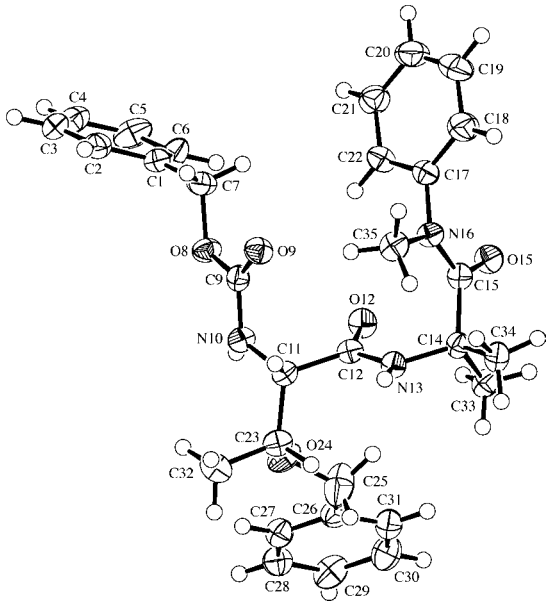


Figure 13. *ORTEP Plot* [86] *of the Molecular Structure of* **38** (50% probability ellipsoids; arbitrary numbering of the atoms)

The whole synthesis of the protected 6-16 segment **4** is resumed in *Figure 14*. This overview shows that the yields are good to very good, except in some cases with the TBTU/HOBt coupling method. The introduction of all Aib residues using the 2*H*-azirin-3-amines **1**, **2**, and **3** proved to be ideally suited and was performed in 75% to quantitative yield. The chosen protecting groups allowed the different conversions under acidic and basic conditions in the synthesis.

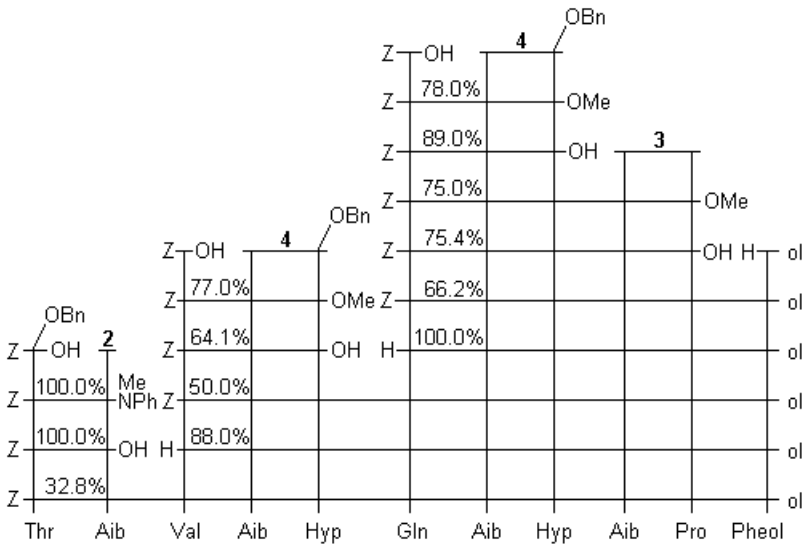


Figure 14. *Synthesis of the Protected 6-16 Segment* **4** *of* Zervamicin II-2

The Z protecting group was removed smoothly, whereas the benzyl protecting groups of the threonine and the two hydroxy proline residues remained stable.

6.3 Conclusion

The protected eleven amino acid 6-16 segment **4** of the peptaibol *Zervamicin II-2* was synthesized by using the azirine/oxazolone method for the introduction of all Aib residues. Whereas a 2,2-dimethyl-2*H*-azirin-3-amine was used as the building block for Aib, methyl 2,2-dimethyl-2*H*-azirine-3-prolinate and -3-(3-hydroxyprolinate) proved to be ideally suited as dipeptide synthons for the introduction of Aib-Pro and Aib-Hyp, respectively. The coupling of Z-protected amino acids or peptide acids with the 2*H*-azirin-3-amines were performed in 75% to quantitative yield.

7 *Hypomurocins A1, A3* and *A5*

7.1 Introduction

The *Hypomurocin* A family belongs to the SF4 peptaibol class [38], which is the one with the less collected data about structure and conformation. The members of this family are characterized by 11 to 14 residues and a Gln or an Asn residue in position 2, they contain neither aromatic nor charged residues but two Pro residues at the same place for all of the 11-residue molecules.

The total synthesis of HM A1 (**5**, 1-(*N*-acetyl-2-methylalanyl-L-glutaminyl-L-valyl-L-valyl-2-methylalanyl-L-prolyl-L-leucyl-L-leucyl-2-methylalanyl)-*N*-[(1*S*)-1-(hydroxymethyl)-3-methylbutyl]-L-prolinamide), HM A3 (**6**, 1-(*N*-acetyl-2-methylalanyl-L-glutaminyl-L-valyl-L-leucyl-2-methylalanyl-L-prolyl-L-leucyl-L-isoleucyl-2-methylalanyl)-*N*-[(1*S*)-1-(hydroxymethyl)-3-methylbutyl]-L-prolinamide) and HM A5 (**7**, 1-(*N*-acetyl-2-methylalanyl-L-glutaminyl-L-isoleucyl-L-isoleucyl-2-methylalanyl-L-prolyl-L-leucyl-L-leucyl-2-methylalanyl)-*N*-[(1*S*)-1-(hydroxymethyl)-3-methylbutyl]-L-prolinamide) is described here. These three peptaibols are representatives of the 12 different *Hypomurocins* of the HM family, which is divided into the subgroups of HM A (11 amino acids) and HM B (18 amino acids, *Figure 15*). These peptaibols have been isolated from the ascomycetous fungus *Hypocrea muroiana* Hino et Katsumoto using reverse phase HPLC (RP-HPLC) and their structures have been characterized by GC-MS analysis [88].

```
Ac-Aib-Gln-Val-Val-Aib-Pro-Leu-Leu-Aib-Pro-Leuol       HM A1
Ac-Iva-Gln-Val-Val-Aib-Pro-Leu-Leu-Aib-Pro-Leuol       HM A2
Ac-Aib-Gln-Val-Leu-Aib-Pro-Leu-Ile-Aib-Pro-Leuol       HM A3
Ac-Aib-Gln-Ile-Val-Aib-Pro-Leu-Leu-Aib-Pro-Leuol       HM A4
Ac-Aib-Gln-Ile-Ile-Aib-Pro-Leu-Leu-Aib-Pro-Leuol       HM A5
Ac-Aib-Gln-Ile-Leu-Aib-Pro-Leu-Ile-Aib-Pro-Leuol       HM A5a

Ac-Aib-Ser-Ala-Leu-Aib-Gln-Aib-Val-Aib-Gly-Aib-Aib-Pro-Leu-Aib-Aib-Gln-Valol HM B1
Ac-Aib-Ser-Ala-Leu-Aib-Gln-Aib-Val-Aib-Gly-Aib-Aib-Pro-Leu-Aib-Aib-Gln-Leuol HM B2
Ac-Aib-Ala-Ala-Leu-Aib-Gln-Aib-Val-Aib-Gly-Aib-Aib-Pro-Leu-Aib-Aib-Gln-Valol HM B3a
Ac-Aib-Ser-Ala-Leu-Aib-Gln-Iva-Val-Aib-Gly-Aib-Aib-Pro-Leu-Aib-Aib-Gln-Valol HM B3b
Ac-Aib-Ser-Ala-Leu-Aib-Gln-Aib-Val-Aib-Gly-Iva-Aib-Pro-Leu-Aib-Aib-Gln-Valol HM B4
Ac-Aib-Ser-Ala-Leu-Aib-Gln-Aib-Val-Aib-Gly-Iva-Aib-Pro-Leu-Aib-Aib-Gln-Leuol HM B5
```

Figure 15. *Sequences of the 12* Hypomurocins (Ac = acetyl; Leuol, Valol = amino alcohol at the C-terminus)

It is to be expected that the conformation of HM A1 (**5**), A3 (**6**) and A5 (**7**) is helical due to the presence of several Aib moieties, as it is known for other peptaibols. Because the HM A sequences contain two Aib-Pro units, they were attractive targets for the application of the azirine/oxazolone method by using the Aib-Pro synthon **2**. As usual, we used the Z protecting group to allow transformations under basic and acidic conditions, and the TBTU/HOBt coupling was used for segment condensations. Furthermore, a structural study of HM A1, A3 and A5 by NMR spectroscopy and X-ray crystallography was performed.

7.2 Synthesis

7.2.1 Strategy of the synthesis

As this part of the work constitutes a project to synthesize HM A1, A3 and A5 and to compare their structures, the first requirement before starting the syntheses is a good preparative protocol and an efficient strategy to minimize the number of steps for all three syntheses. For this purpose, two questions had to be answered:

- is the number of steps in the syntheses depending on the way the molecules are synthesized?
- what are the longest common sequences of HM A1, A3 and A5?

For the first question, a mathematical expression (*Equation 1*) was empirically set up to calculate the number of steps for the synthesis of a peptaibol :

$$S = 2\times L - 2\times N - 3 \quad (1)$$

L = Length in units of the peptaibol (*e.g.* for HM A1, L = 11).

N = Number of bi-moieties of Aib-X (X = Pro, Hyp,…) being introduced by the azirine/oxazolone method. In our case, X = Pro and N = 2.

S = Number of steps to synthesize the peptaibol with the azirine/oxazolone method.

As it is shown in *Equation 1*, the number S does not depend on the way the synthesis is performed, but only on how many bi-moieties N can be introduced by the azirine/oxazolone method, as well as the length L of the peptaibol. Thus for HM A1, A3 or A5, the required number of steps is the same and is based on L = 11 and N = 2 giving a result of S = 15 steps. So for the three syntheses, we have "in the worst case" a total of 45 steps to carry out.

With the answer of the second question, we will considerably reduce this number. Let's compare the common segments of our three targets (*Figure 16*). We notice that the three peptaibols have the same sequence Ac-Aib-Gln, at the N-terminus.

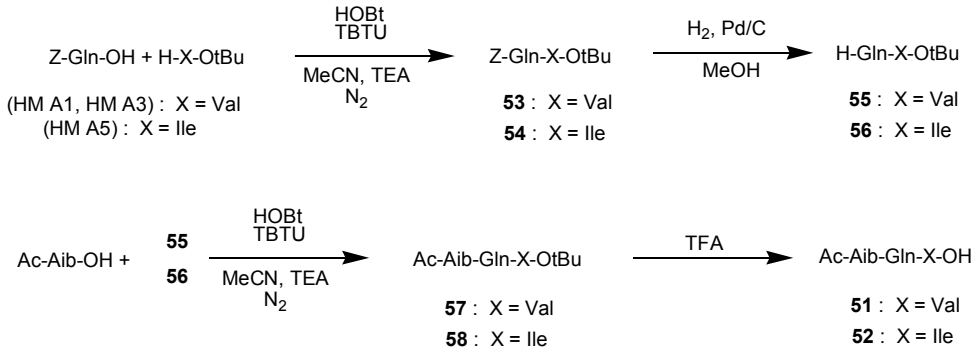
```
   1   2   3   4   5   6   7   8   9  10   11

Ac-Aib-Gln-Val-Val-Aib-Pro-Leu-Leu-Aib-Pro-Leuol     HM A1 (5)
Ac-Aib-Gln-Val-Leu-Aib-Pro-Leu-Ile-Aib-Pro-Leuol     HM A3 (6)
Ac-Aib-Gln-Ile-Ile-Aib-Pro-Leu-Leu-Aib-Pro-Leuol     HM A5 (7)
```

Figure 16. *Common Sequences of HM A1, A3 and A5*

The first idea would be to prepare the dipeptide Ac-Aib-Gln-OH (**50**) by coupling Ac-Aib-OH and H-Gln-OtBu and deprotecting the C-terminus of the resulting molecule. The subsequent unknown coupling step between **50** and H-Val-OtBu (for HM A1 and A3) or H-Ile-OtBu (for HM A5) might result in a substantial loss of material as Gln bears a rather big side chain. As Ac-Aib-OH is either very expensive commercially or has to be prepared by acetylating H-Aib-OH, a reaction which can be performed in low yield only, we decided to introduce Aib in position 1 of the tripeptides Ac-Aib-Gln-Val-OH (**51**) and Ac-Aib-Gln-Ile-OH (**52**) in the last step because the commercial

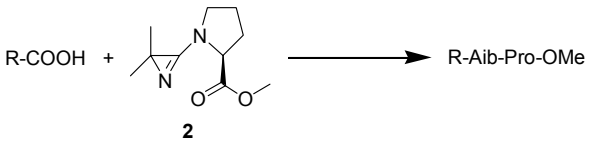
starting materials for Gln, Val and Ile are cheap. Moreover, the tripeptide Ac-Aib-Gln-Val-OH (**51**) can be used for the synthesis of both HM A1 and A3. Thus, Z-Gln-OH must be coupled first with H-Val-OtBu resp. H-Ile-OtBu; the *tert*-butyl protection was chosen to allow a smooth deprotection with TFA and to avoid the cleavage of the acetyl group on Aib. The obtained products Z-Gln-Val-OtBu (**53**) and Z-Gln-Ile-OtBu (**54**) should be deprotected to give H-Gln-Val-OtBu (**55**) and H-Gln-Ile-OtBu (**56**), respectively, coupled with Ac-Aib-OH to lead to the two tripeptides **57** and **58**, and deprotected with TFA to yield **51** and **52** (*Scheme 11*).

Scheme 11. Strategy for the Synthesis of the Segments 1-3 **51** *and* **52**



The inspection of the rest of the three peptaibols in *Figure 16* reveals that two Aib-Pro sequences in positions 5-6 and 9-10 are in common, which should be introduced by the azirine/oxazolone method at a C-terminal peptide or amino acid by using methyl *N*-(2,2-dimethyl-2*H*-azirin-3-yl)-L-prolinate **2** (*Scheme 12*):

*Scheme 12. Reaction of the 2*H*-Azirin-3-amine* **2**



2

So, the following tripeptide sequences have to be prepared: Val-Aib-Pro, Leu-Aib-Pro, and Ile-Aib-Pro. Whereas Val-Aib-Pro is only a segment of HM A1, Leu-Aib-Pro is a common sequence in both HM A1 (residues 8-9-10) and HM A3 (residues 4-5-6), which allows to use this segment in both syntheses. Moreover, HM A1 and HM A5 contain the same C-terminal pentapeptide Leu-Leu-Aib-Pro-Leuol (residues 7-11). This will help to reduce the number of reaction steps. Finally, HM A3 and HM A5 contain the common tripeptide sequence Ile-Aib-Pro (residues 8-9-10 for HM A3 and 4-5-6 for HM A5). As the sequence Aib-Pro should be introduced at the very beginning of the synthesis, a large amount of methyl *N*-(2,2-dimethyl-2*H*-azirin-3-yl)-L-prolinat has to be prepared. This was not a real problem because, firstly, its synthesis is well known, and secondly, its synthesis could be optimized significantly.

The complete strategy for the syntheses is shown in *Figure 17* on the next page. The common parts of HM A1, A3 and A5 are highlighted.

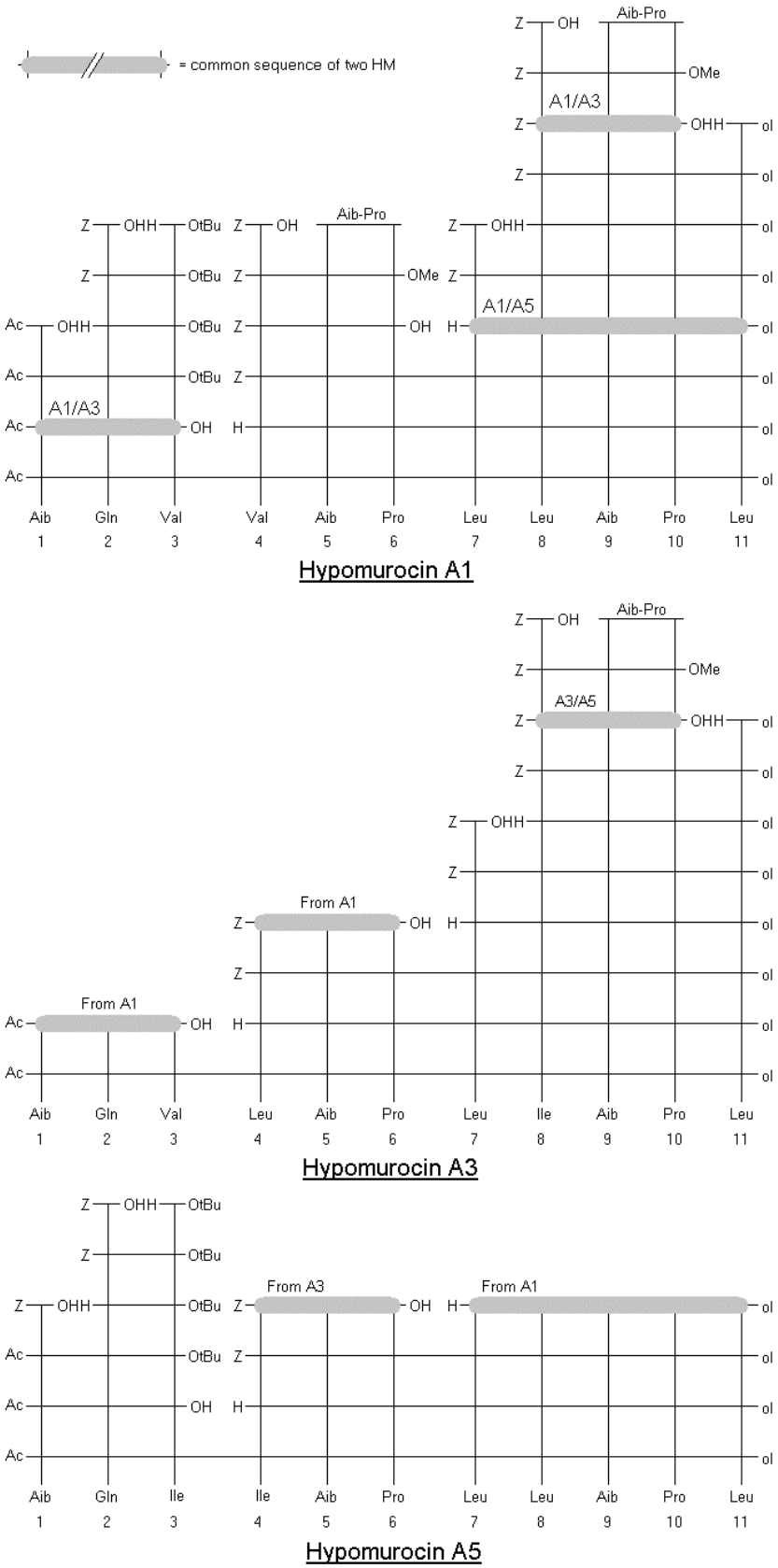


Figure 17. *Overview of the Syntheses of HM A1, A3 and A5*

We can see that the total number of steps for the synthesis of the three HM sequences can be reduced to 31 which is about 2/3 of the initial number of 45. With the goal of receiving 200 mg of each target molecule, a "backward calculation" (*Figure 18*) with an hypothetical "pessimistic" yield for each step (Protocol A: 50-70% according to the size of the peptide or amino acids to be coupled; protocol B: 70 %; protocol C: 70 %; protocol D: 70-85% according to the size of the peptide), was performed.

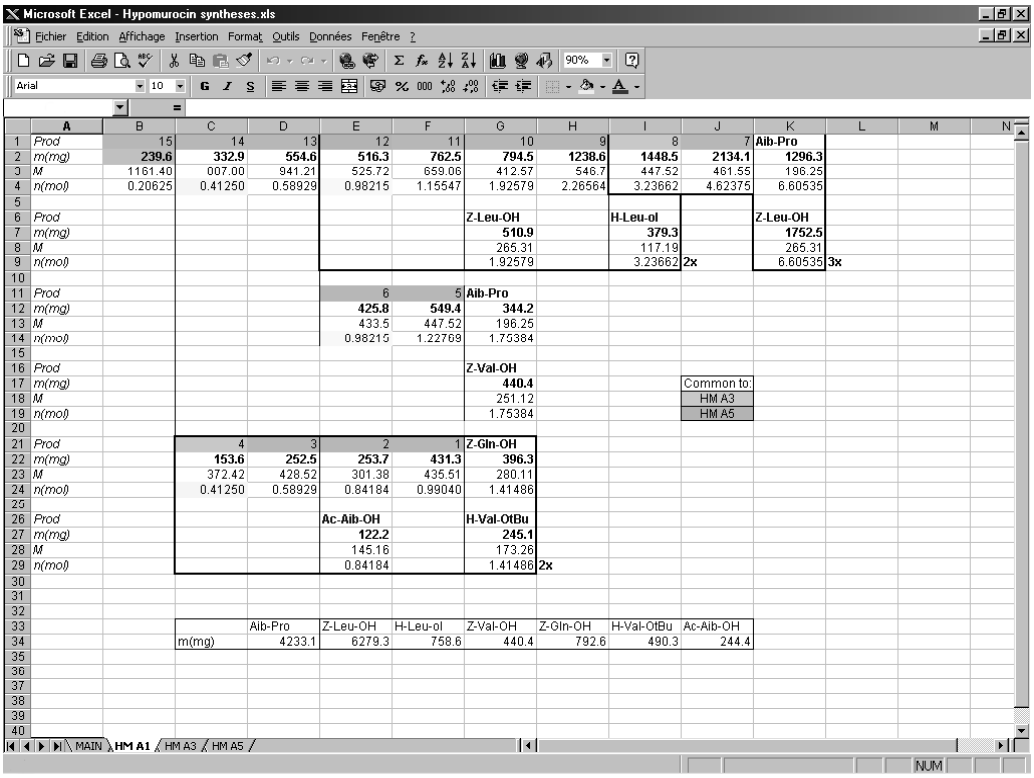


Figure 18. *Calculation of the needed Amount of Material for the Synthesis of HM A1 (calculations for HM A3 and HM A5 are similar)*

In accordance with our negative experience by coupling two long sequences in the synthesis of the *Zervamicin II-2* segment [52], we decided to prepare the three *Hypomurocins* from the three segments 1-3, 4-6 and 7-11.

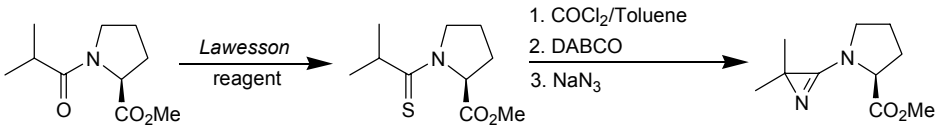
7.2.2 Preparation of the 2*H*-Azirin-3-amine 2

As a relatively large amount of methyl *N*-(2,2-dimethyl-2*H*-azirin-3-yl)-L-prolinat was needed, an attempt to optimize the synthesis was made. The following reactions were optimized for the synthesis of **2**. These considerations should be applicable for the synthesis of any 2*H*-azirin-3-amine, as long as a solution of $COCl_2$ in toluene is used (in contrast to the synthesis of **2** and **3** by using $COCl_2$ gas [51]), and for the 2*H*-azirin-3-amine, which does not contain the hydroxyproline unit in its second position, to avoid the formation of **36** (see chapter 6.2.2 [53]).

The synthesis of the needed amount of **2** for the introduction of the sequence Aib-Pro was prepared according to [51], and the desired quantity was estimated on the basis of reported yields. In [51], the overall yield of **2** from methyl *N*-(2-

methylpropanoyl)-L-prolinate (**59**) is reported to be *ca*. 30% (*i.e.* **59** → methyl *N*-(2-methylpropanthioyl)-L-prolinate (**60**; 45 min reaction time, purification by flash column chromatography on SiO_2 and distillation, 60%) and **60** → **2** (purified by two subsequent flash column chromatographies on SiO_2, 52%)). Actually, we could considerably optimize these procedures resulting in a reduction of time, costs and chemicals. In the present work, the thionation of **59** to lead to **60** was carried out overnight and gave **60** in 93% yield (a small extract of the crude material was purified to determine the yield) without any side reaction (*Scheme 13*).

Scheme 13. Thioamide Intermediate **60** *in the Synthesis of* **2**



59 **60** **2**

The thioamide **60** was filtered through a *Celite* pad, whereupon the color of the mixture turned from deep brown to pale yellow. A thin layer chromatography of the mixture indicated that only two very minor impurities remained, and the product was used without further purification for the next step. At *ca*. 0°, **60** crystallized from the crude mixture to give crystals that were suitable for an X-ray crystal-structure determination (*Figure 19*).

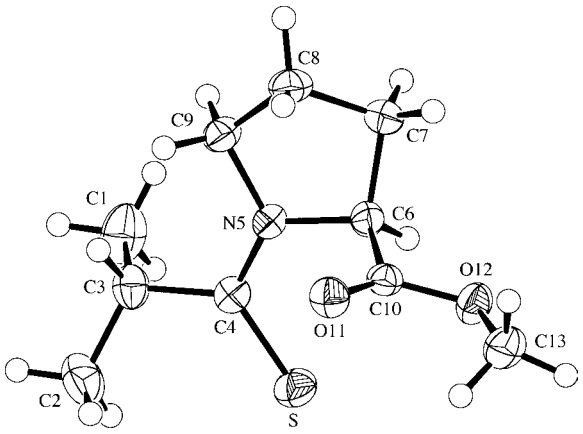


Figure 19. *ORTEP Plot* [86] *of the Molecular Structure of the Thioamide* **60** (50% probability ellipsoids; arbitrary numbering of the atoms)

The crystal of **60** was enantiomerically pure, and the absolute configuration of the molecule has been determined independently by the diffraction experiment, establishing that the molecule has the expected (6*S*)-configuration.

As it is known that 2*H*-azirin-3-amines frequently decompose on SiO_2, it was decided to avoid the purification and to use the crude material for the peptide synthesis. With the aim of determining the yield of the synthesis of **2**, the coupling reaction with Z-Val-OH to give Z-Val-Aib-Pro-OMe (**61**), the yield of which has been reported [51], was carried out. With the assumption of an 86% yield for the coupling reaction [51], the purity of **2** could be estimated as *ca*. 80%. Therefore, the overall yield of the synthesis of **2** from **59** was *ca*. 70% *i.e.*, *ca.* 40% higher than reported for the previous synthesis.

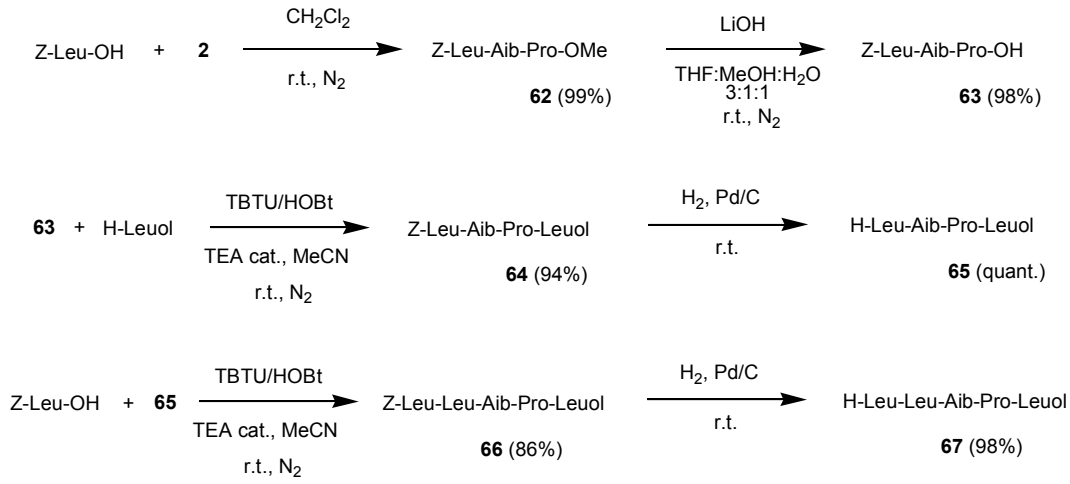
Of course, after the coupling reaction, the crude **61** contains the impurities of the thioamide and azirine syntheses. But there has been no evidence of side effects or alteration of the product due to these impurities, and, while it was not possible to purify **2** without significant loss of material, the tripeptide **61** could be easily purified by flash chromatography.

In conclusion, the reaction time in the thioamide synthesis can be extended to 15 h without undesired side effects leading to an increase of the yield more than twice. Furthermore, no purification of the 2*H*-azirin-3-amine **2** is necessary, thus avoiding the loss of material during chromatography. The purity of **2** is about 80%, and the dragged impurities are easily separable by flash column chromatography after the first coupling with **2**.

7.2.3 *Hypomurocin A1*

For the coupling of the segments and/or amino acids, the TBTU/HOBt method was used successfully. The Z group was chosen to protect the N-terminus of peptides and amino acids, and its deprotection was performed under an H_2 atmosphere with Pd/C (5%). The hydrolysis of methyl esters was achieved with LiOH (3 equiv.) in THF/MeOH/H_2O 3:1:1 overnight, and the *tert*-butyl ester was cleaved with TFA in 90 min.

Scheme 14. Synthesis of the Pentapeptide **67**

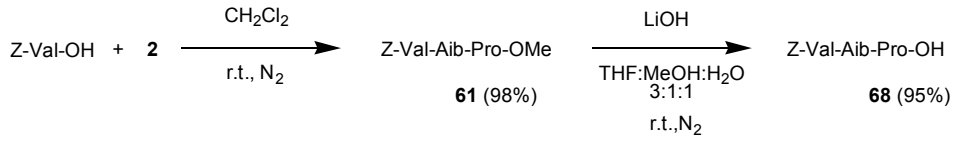


The synthesis started with the coupling of Z-Leu-OH with the 2*H*-azirin-3-amine **2** in CH_2Cl_2 at room temperature overnight under an N_2-atmosphere to give the tripeptide **62** in 99% yield (*Scheme 14*). The latter was hydrolyzed to give **63** in 98% yield. Until this step, the synthesis was run in triple quantity as **63** will be used in the syntheses of HM A1, HM A3, and HM A5. A convenient amount of **63** was put aside for the synthesis of HM A3. The hydrolyzed tripeptide **63** was coupled with the α-amino alcohol H-Leuol in 94% yield. The resulting tetrapeptide **64** was quantitatively deprotected under an H_2 atmosphere (Pd/C) to give **65**, which was coupled again with Z-Leu-OH to give **66** in 86% yield. Deprotection of the latter gave the pentapeptide **67** in 98% yield, being the first major segment in the synthesis of HM A1. Here again, a

convenient amount of the pentapeptide **67** was set aside for the synthesis of HM A5. No particular problem was met in this part of the synthesis.

The synthesis of the segment 4-6 (Z-Val-Aib-Pro-OH; **68**) of HM A1 was carried out in analogy to that of the segment 7-11 by the coupling reaction of Z-Val-OH with **2**, followed by the hydrolysis of the resulting product **61** (*Scheme 15*). It is worth mentioning that the coupling reaction occurred with the surprisingly high yield of 98% by using 1.2 equiv. of **2**.

Scheme 15. Synthesis of the Tripeptide **68**



Suitable crystals of **68** for an X-ray crystal-structure determination were grown from MeOH (*Figure 20*). The asymmetric unit contains one molecule of **68** and disordered solvent molecules, which are estimated to be one H_2O and one MeOH molecule. Both NH groups are involved in intermolecular H-bonds.

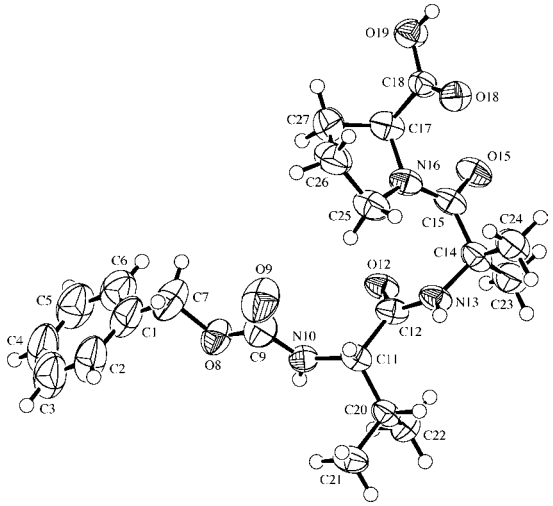


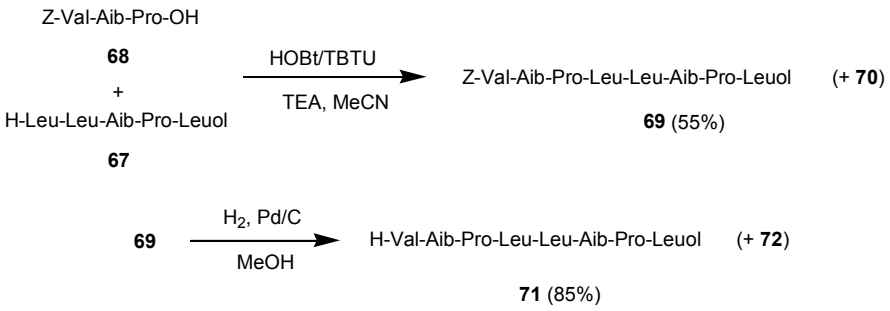
Figure 20. *ORTEP Plot* [86] *of the Molecular Structure of the Tripeptide* **68** (50% probability ellipsoids; arbitrary numbering of the atoms)

The amide H-atom of Val^1 forms an intermolecular H-bond with the C-terminal carbonyl O-atom of a neighboring molecule. The amide H-atom of Aib^2 forms an intermolecular H-bond with the carbonyl O-atom of the Aib^2 of a different neighboring molecule. The combination of these interactions links the peptide molecules into a two-dimensional network. The terminal COOH group probably forms a H-bond to a H_2O molecule, as a H···O distance of about 1.8 Å was detected during attempts to model the solvent molecules.

The first critical step in the synthesis of HM A1 **5** was expected to be the coupling of the two segments **68** and **67**. The TBTU/HOBt coupling led to the

octapeptide **69** in 55% yield (*Scheme 16*) [1]). This relatively low yield could indicate that the conformation of the two peptides is somehow hindered, resulting in a difficult coupling. The deprotection of the octapeptide **69** to give **72** was performed in 85% yield.

Scheme 16. Synthesis of the Octapeptide **71**



It is usually quite difficult to obtain good crystals of linear peptides, but, in the case of **69**, beautiful long needle crystals were obtained, so an X-ray crystal-structure determination was carried out (*Figure 21*).

The space group permits the compound in the crystal to be enantiomerically pure, but the absolute configuration of the molecule was not determined. The enantiomer used in the refinement was based on the expected (*S*)-configuration of all peptide units in the molecule, and the structure determination confirmed that no unexpectedly inverted peptide units are present (*cf.* the (*R*)-proline unit in **72**). The asymmetric unit contains one molecule of **69** plus one half of a molecule of AcOEt, which is disordered about a C_2 axis. The terminal *i*-Pr atoms of both Leu moieties are disordered over two conformations. The peptide molecules produce a complex pattern of inter- and intramolecular H-bonds, although the intermolecular interactions merely link the molecule into extended chains such that the N termini always point in the same direction (*Figure 23*). The Leuol[8] OH group forms an intramolecular H-bond with the Pro[7] carbonyl O-atom. The amide H-atom of Val[1] forms an intermolecular H-bond with the hydroxy O-atom of Leuol[8] of a neighboring peptide molecule, while the amide H-atom of Aib[2] is H-bonded to the carbonyl O-atom of Pro[7]. The amide H-atoms of Leu[4], Leu[5] and Leuol[8] form intramolecular H-bonds with carbonyl O-atoms such that the corresponding β-turns are formed. Consecutive segments of *i,i+3* hydrogen-bonded elements encode for the 3_{10}-helix, which therefore presents the dominating secondary structural element in this peptide. In addition, the amide H-atom of Aib[6] is involved in a π-turn.

The molecular packing of **69** clearly shows the typical S-shape structure of the helices. There are no intermolecular H-bonds between the polypeptide chains. The disorder in some peptide side groups and the presence of AcOEt molecules in the crystal have little influence on the S-shape structure, and there are no H-bonds between

[1]) The mixture, which was obtained after the coupling reaction, showed two different spots on the TLC. After their separation and NMR characterisation, the main spot proved to be the expected product **69**, and the second one was very close to **69**. The MS of this minor product **70** proved that it had the same molecular weight as **69**. Nevertheless, this minor product was disregarded.

the solvent and the helical peptide **69**. This type of self-association is known for other peptaibols, like, *e.g.*, *Trichotoxin A50E* [38], which form transmembrane ion channels.

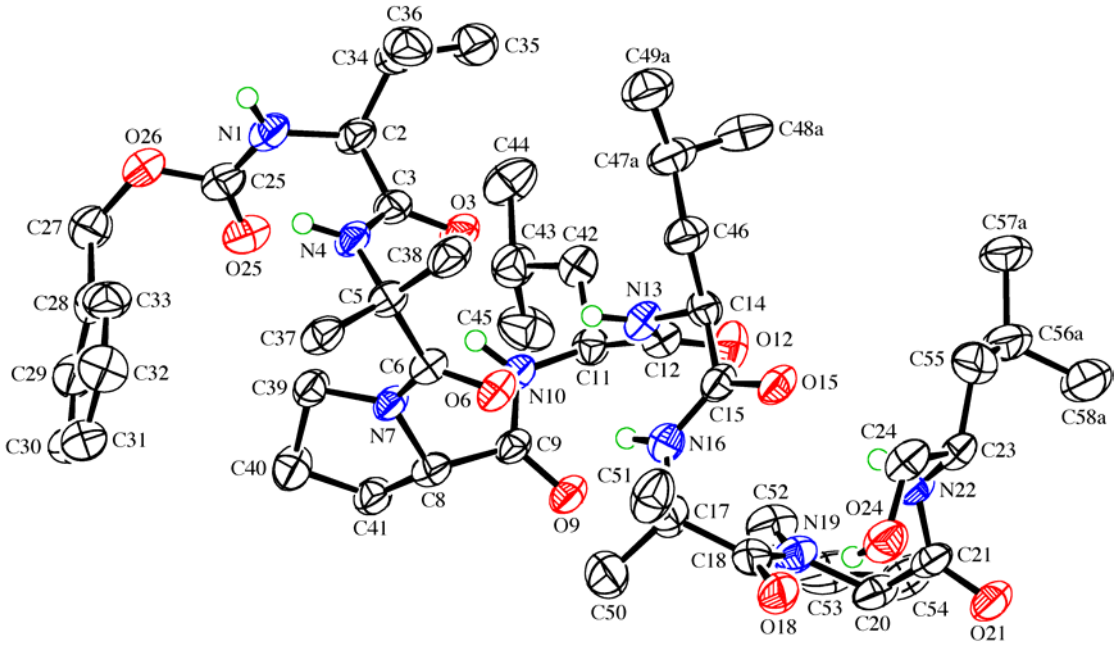


Figure 21. *ORTEP Plot* [86] *of the Molecular Structure of* **69** (conformation A; 50% probability ellipsoids; arbitrary numbering of the atoms)

The superimposition of the two conformations of the peptide molecule **69** shows their similarity (*Figure 22*).

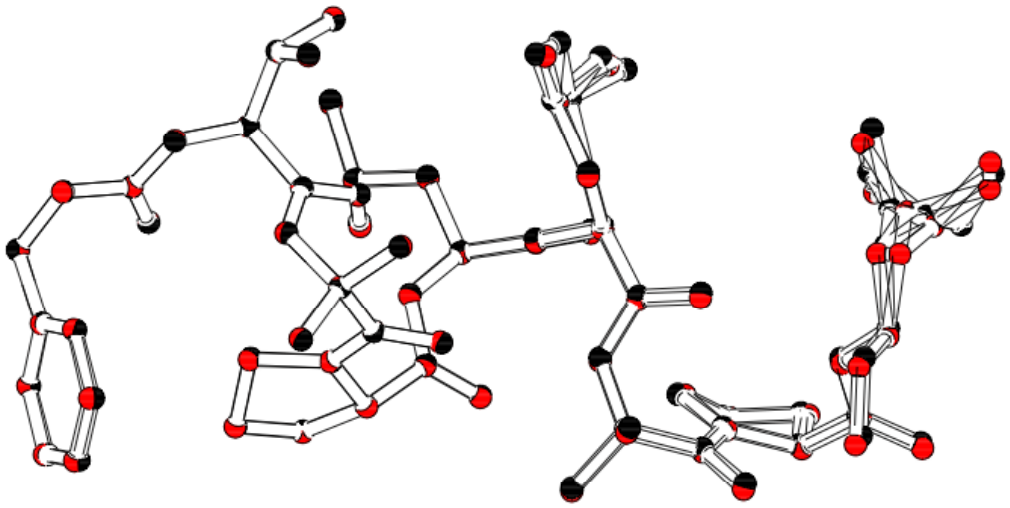


Figure 22. *Superimposition of the two Conformations A and B of the Molecular Structure of* **69**

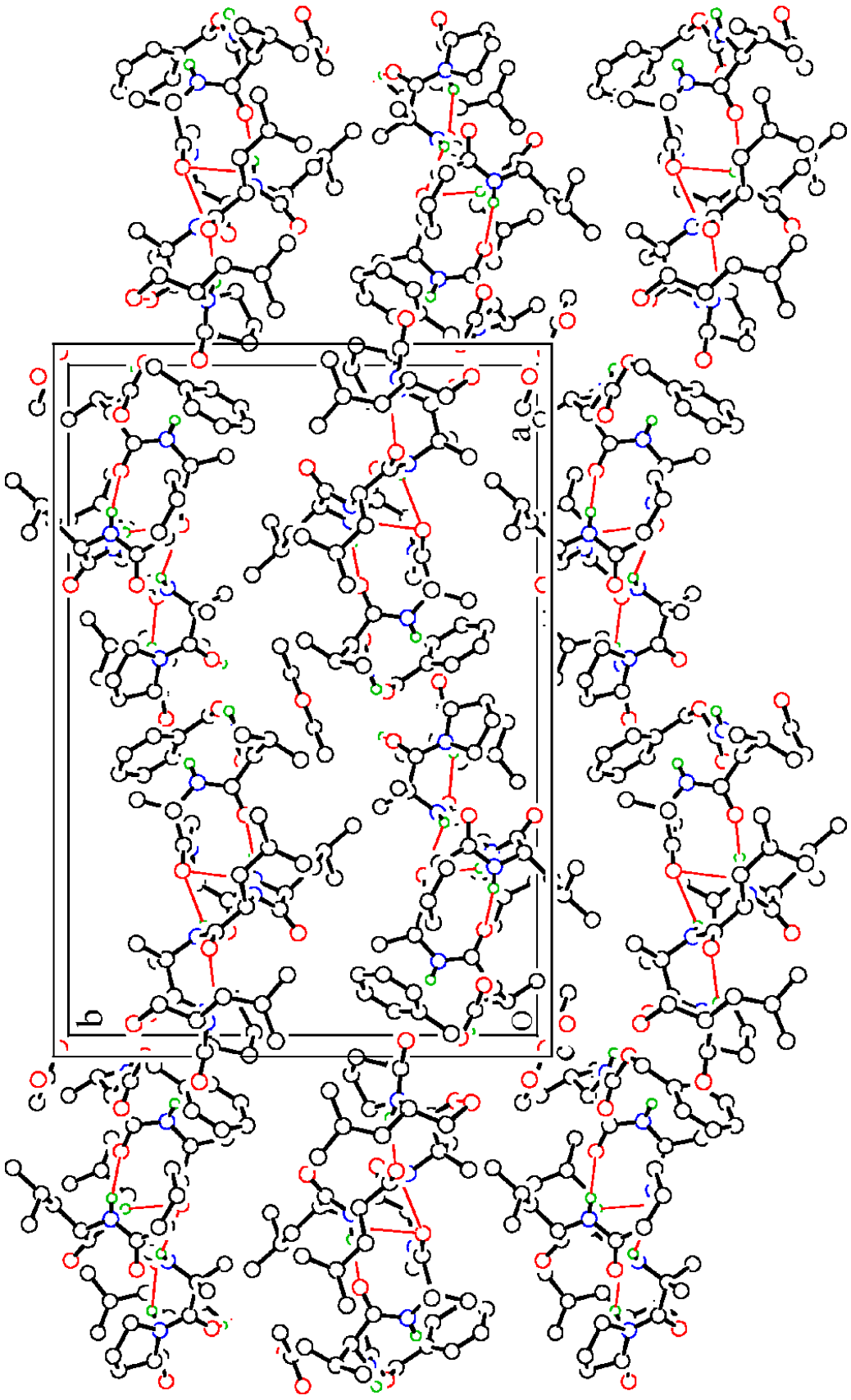


Figure 23. *Molecular Packing of* **69** *projected down the* b*-Axis showing the H-Bonding Scheme* (equivalent isotropic spheres for atoms; uninvolved H-atoms omitted for clarity; color code: C-atoms: black, O-atoms: red, N-atoms: blue, H-atoms: green, and H-bonds: red)

The next step in the synthesis was the deprotection of the N-terminus of **69**, *i.e.* the hydrogenolytic cleavage of the Z group. The mixture showed again two spots on the TLC, and two products, **71** and **72**, were isolated. The NMR characterization established that **71** (R_f(B) = 0.06) was the expected product.

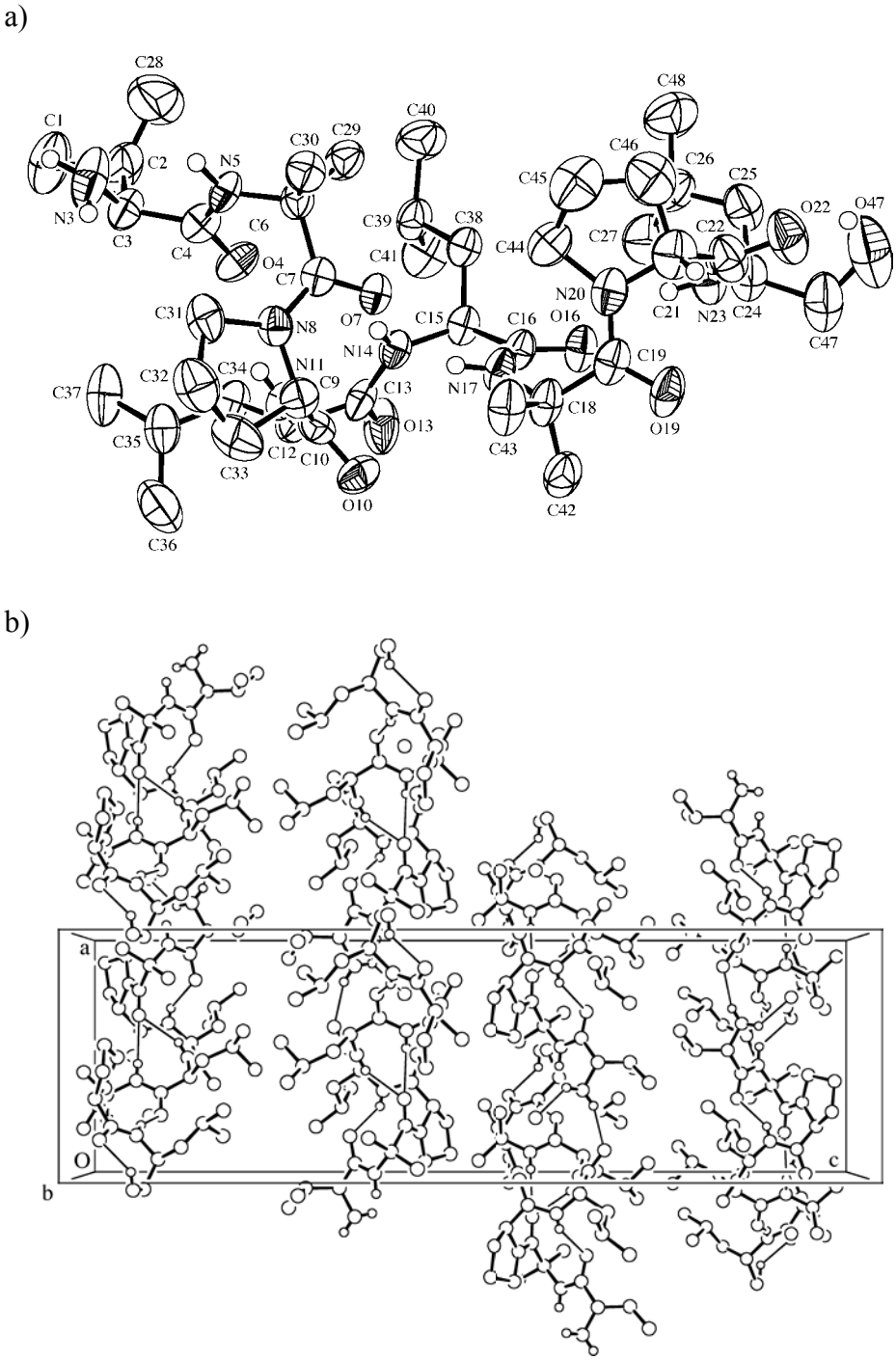


Figure 24. *a) ORTEP Plot* [86] *of the Molecular Structure of the Octapeptide* **72** (50% probability ellipsoids; arbitrary numbering of the atoms)*; b) The Molecular Packing Projected down the* b-*Axis showing the H-Bonding Scheme* (equivalent isotropic spheres for atoms; uninvolved H-atoms omitted for clarity)

The second one, **72** (R_f(B) = 0.24), has again a structure very similar to that of **71**. The crystal structure of **72** was determined (*Figure 24*) and proved unequivocally that **72** was the epimer of **71** with a (*R*)-configured Pro[10] [2]).

The space group permits the compound **72** in the crystal to be enantiomerically pure, but the absolute configuration of the molecule has not been determined. The enantiomer used in the refinement was based on the presumed (*S*)-configuration for the majority of the peptide units of the molecule. Based on this assumption, the molecule has the (3*S*, 9*S*, 12*S*, 15*S*, 21*R*, 24*S*)-configuration. The asymmetric unit contains one molecule of the peptide plus one site which is 50% occupied by a H_2O molecule. The intermolecular interactions link the peptide and H_2O molecules into two-dimensional networks. Each NH group of the molecule, except for the amide H-atom of Aib[2], acts as a donor for H-bonds. The NH_2 group forms two intermolecular H-bonds. One interaction is with the O-atom of the H_2O molecule, while the second interaction is with the carbonyl O-atom of Leu[4] of a neighboring molecule. Although the H-atoms of the H_2O molecule could not be located reliably, the H-bond donor interactions of the H_2O molecule can be estimated from suitable O···O distances. The H_2O molecule donates one H-bond to the carbonyl O-atom of Leu[4] (O···O = 2.91(1) Å) of **72** in the same asymmetric unit, and a second H-bond to the carbonyl O-atom of Pro[7] of a different neighboring peptide molecule (O···O = 2.68(1) Å). The combination of all intermolecular interactions generates a two-dimensional network of peptide and H_2O molecules. Aside from the amide H-atom of Aib[2], the NH and OH groups form intramolecular H-bonds. Similar to **69,** the amide H-atoms of Leu[4], Leu[5] and Leuol[8] form intramolecular hydrogen bonds which result in the corresponding β-turns, whereas the amide proton of Aib[8] is involved in a π-turn. The Leuol[8] OH group forms an intramolecular H-bond with the Pro[7] carbonyl O-atom. Again, the 3_{10} helix is the dominating secondary structural element in this peptide.

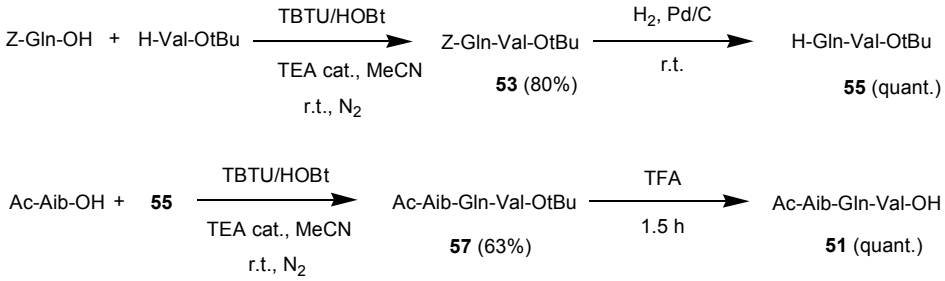
According to the crystal structure of **72**, in which the Pro[10] residue has the (*R*)-configuration, we conclude that **70** and **72** are the respective epimers of **69** and **71** with (*R*)-Pro[10]. The only explanation of this surprising result is that an epimerization had occurred either during the saponification **62** → **63** or the coupling of H-Leuol with **63** (*Scheme 14*), although at this stage of the synthesis, no epimerization was detected. However, Pro residues are not expected to readily undergo racemization as reported in the literature [73]. Moreover, the amounts of isolated **70** and **72** were very small and represent *ca*. 1–2% yield. This epimerization shows that the introduction of the Leuol unit into the peptide chain of HM A1 is a sensitive step. Therefore, it is advantageous to carry out this coupling as early as possible in the synthesis.

The synthesis of the last segment Ac-Aib-Gln-Val-OH (**51**) of HM A1 was performed by coupling Z-Gln-OH with H-Val-OtBu under the usual coupling conditions with 80 % yield (*Scheme 17*). It was very difficult to purify the dipeptide Z-Gln-Val-OtBu (**53**) as it has a R_f value close to those of the coupling or additive reagents. Furthermore, the dipeptide was like a kind of plaster powder and was difficult to dissolve in polar solvents like MeOH, MeCN. This dipeptide was deprotected at the

[2]) Based on this result, we propose that the above mentioned minor product **70**, which has been proved to be an isomer of **69** (*Scheme 16*), is the corresponding epimer with an (*R*)-configured Pro residue. Most likely, **72** is the product of the hydrogenation of **70** as an impurity of **69** (see below).

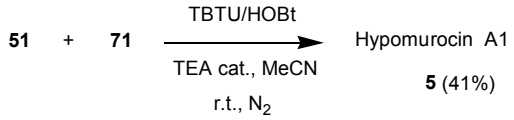
N-terminus, leading quantitatively to **55,** which is a very viscous oil. The latter was coupled with Ac-Aib-OH [26] under the usual conditions to give the tripeptide **57** in only 63% yield, illustrating again the effect of steric hindrance due to the presence of Aib. Finally, the *tert*-butyl ester **57** was cleaved with TFA to give **51** quantitatively. According to our strategy, this last major sequence was synthesized in double amount and a convenient portion was put aside for the synthesis of HM A3.

Scheme 17. Synthesis of the Tripeptide **51**



The last step of the synthesis of HM A1 was the coupling of the tripeptide **51** and the octapeptide **71** (*Scheme 18*). Under the chosen conditions (TBTU/HOBt), the yield of **5** was again low. This can be explained by a hindered spatial conformation due to the generally observed rigid helical conformation of peptaibols.

*Scheme 18. Last Coupling Reaction to give HM A1(***5***)*

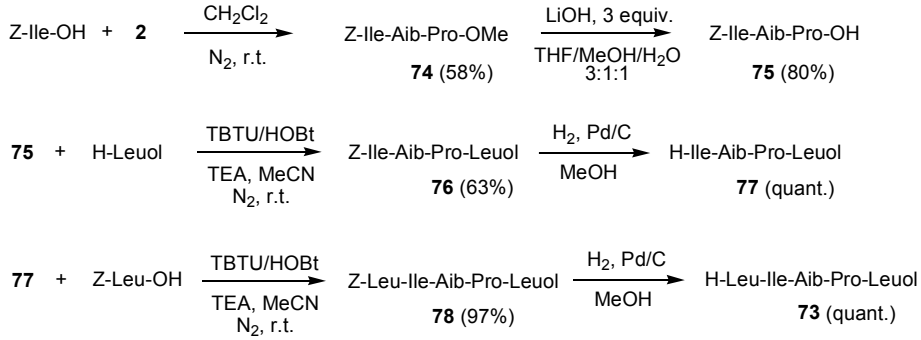


7.2.4 ***Hypomurocin A3***

The synthesis of HM A3 started by synthesizing the unprotected segment 7-11, *i.e.* H-Leu-Ile-Aib-Pro-Leuol (**73**, *Scheme 19*). The reaction of Z-Ile-OH with the 2*H*-azirin-3-amine **2** gave the protected N-terminal tripeptide methyl ester **74** in 58% yield. This low yield is unusual for a 2*H*-azirin-3-amine coupling and can be explained by the partial decomposition of **2** in the crude fraction, which was kept for over one year before use. The ester **74** was hydrolyzed with LiOH to give **75** in 80% yield. Until here, the synthesis had to be run in double quantity according to our strategy. Half of the amount of **75** was set apart for the synthesis of HM A5, and the synthesis of HM A3 was continued with the other half. The tripeptide Z-Ile-Aib-Pro-OH **(75)** was coupled with H-Leuol to give the tetrapeptide Z-Ile-Aib-Pro-Leuol (**76**) in 63% yield.

Surprisingly, **76** gave crystals of good quality incredibly fast, which were suitable for an X-ray crystal-structure determination (*Figure 25*). The tetrapeptide even started to crystallize from the crude mixture after the evaporation of the solvent. The

Scheme 19. Synthesis of the Pentapeptide **73**



crystal structure shows that the hydroxy group and the adjacent amide H-atom of Leuol[4], form intramolecular H-bonds with the carbonyl O-atom of Ile[1] that are seven atoms back along the peptide backbone. This serves to maintain the helical conformation of the peptide.

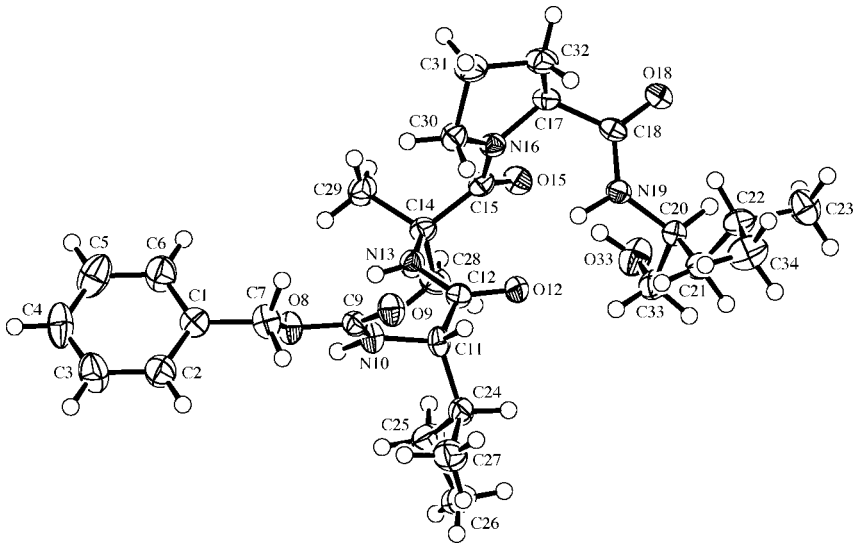


Figure 25. *ORTEP Plot* [86] *of the Molecular Structure of* **76** (50% probability ellipsoids; H-atoms given arbitrary displacement parameters for clarity)

The amide H-atoms of Ile[1] and Aib[2], which are unable to form an intramolecular interaction because of their position in the backbone, form intermolecular H-bonds with the carbonyl O-atom of Pro[3] of a neighboring peptide molecule, although the latter interaction is very weak. These intermolecular interactions link the molecules into extended chains.

The purification of the tetrapeptide **76** by flash column chromatography led to a very small amount of a second product **79**, which was crystallized and its structure resolved by X-ray analysis (*Figure 26*). The structure of **79** proved to be almost identically with that of **76** except for the configuration of the Pro[3] residue, which is *R*. Although peptide couplings with HOBt additives are known to reduce the epimerization, we assume that the introduction of H-Leuol at this position is a weak point in the synthesis as analogous results were obtained in the synthesis of HM A1.

The yields for this coupling reaction are 63% for **76**, and additional 1-2% of **79** were isolated. Consequently, the epimerization occurs only to a small extent.

The X-ray crystal structure analysis of **79** (*Figure 26*) does not allow to draw conclusions for bond lengths or angles that might appear to be unusual because of the poor overall quality of the crystals. The precision of the geometric parameters is also low for the same reason. The space group permitted the compound in the crystal to be enantiomerically pure, but the absolute configuration of the molecule has not been determined. The enantiomer used in the refinement was based on the known *S*-configuration of most chiral centers in the molecule, but the configuration of Pro^3 is *R*. There are two symmetry-independent molecules in the asymmetric unit, which have both the same stereochemistry.

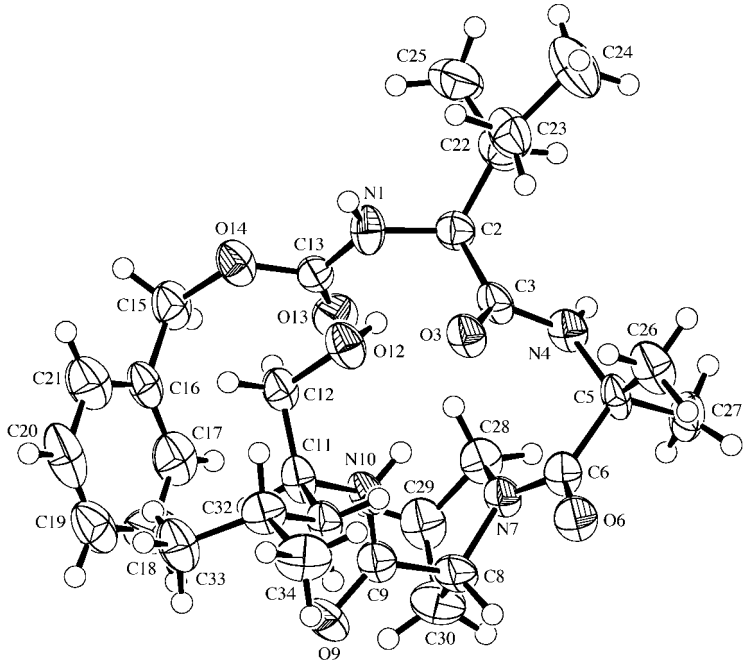


Figure 26. *ORTEP Plot* [86] *of the Molecular Structure of* **79** (50% probability ellipsoids; H-atoms given arbitrary displacement parameters for clarity)

Each hydroxy and amide group of each symmetry-independent molecule **79** acts as a donor for H-bonds. The hydroxy group in each molecule forms an intramolecular H-bond with the amide O-atom of the second peptide unit from the benzyl end of the molecule. The amide H-atom of Ile^1 in molecule A forms an intermolecular H-bond with the hydroxy O-atom of molecule B. In turn, the corresponding amide group of molecule B interacts in the same way with the original molecule A. The amide H-atom of Aib^2 in molecule A forms an intermolecular H-bond with the Aib^2 amide O-atom from an adjacent molecule A and thereby links the A molecules into extended chains. The identical interaction links the B molecules into extended chains which run antiparallel to the chains of A molecules. Finally, the amide H-atom of $Leuol^4$ in molecule A forms an intermolecular H-bond with the amide O-atom of the second peptide unit from the benzyl end of the molecule. The combination of the intermolecular interactions leads to a two-dimensional network of A and B molecules that lies parallel.

The deprotection of **76** with Pd/C under an H_2-atmosphere gave H-Ile-Aib-Pro-Leuol (**77**) quantitatively, from which also suitable crystals for an X-ray structure determination could be obtained (*Figure 27*).

The space group permits the compound **77** in the crystal to be enantiomerically pure, but the absolute configuration of the molecule has not been determined. The enantiomer used in the refinement was based on the known *S*-configuration of Ile[1] in the molecule. All stereogenic centers in the molecule have the *S*-configuration. The asymmetric unit contains two molecules of the peptide, both of which are the same stereoisomer, plus a site for a water molecule that is both partially occupied and disordered about a C_2-axis. Thus, the peptide/H_2O ratio in the crystal is 8:1. The Ile group in one of the independent peptide molecules (molecule B) is disordered over two significantly different conformations. The conformations of molecules A and B also differ significantly in the orientations of the Leuol[4] end of the peptide chain. Each independent peptide molecule displays one intramolecular and several intermolecular hydrogen bonds. The intermolecular interactions link the peptide molecules into a two-dimensional network.

In each of the symmetry-independent peptide molecules **77**, the amide H-atoms of Leuol[4] form an intramolecular H-bond with the Aib[2] amide O-atom that is seven atoms back along the peptide backbone. In molecule A, the terminal amine group forms intermolecular H-bonds with amide O-atoms of a neighboring molecule A (Pro[3]) and a neighboring molecule B (Leuol[4]). The terminal amine group of molecule B only forms one H-bond, that being with the Leuol[4] amide O-atom of another molecule B.

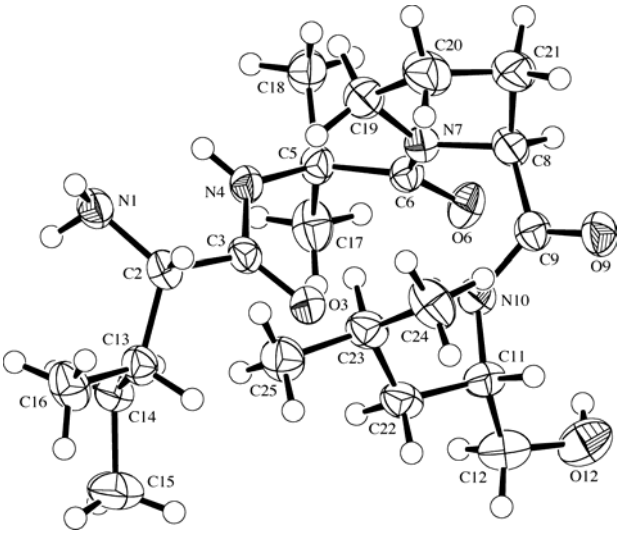


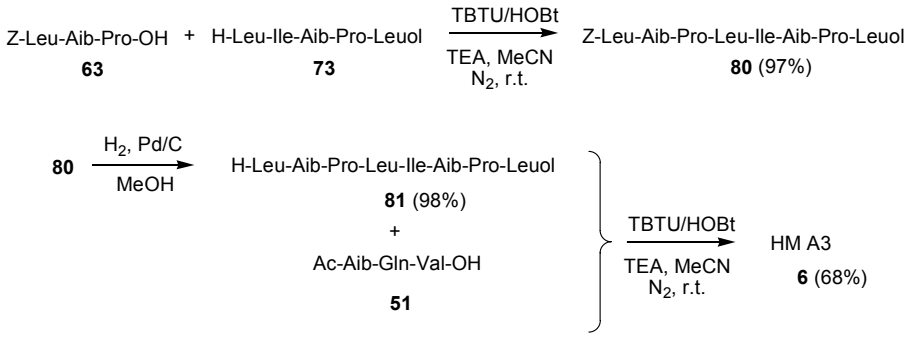
Figure 27. *ORTEP Plot* [86] *of the Molecular Structure A of* **77** (50% probability ellipsoids; H-atoms given arbitrary displacement parameters for clarity)

The amide H-atom of Aib[2] of molecule A forms an intermolecular H-bond with the hydroxy O-atom of the same neighboring molecule A that accepts the interaction from the terminal amine group. This interaction links the A molecules into chains. The amide H-atom of Aib[2] of molecule B forms an intermolecular H-bond with the Leuol[4] amide O-atom of a neighboring molecule A. Together, the intermolecular interactions link the molecules into two-dimensional networks which lie parallel. Although the H-atoms of the H_2O molecule could not be located, the H_2O molecule is close to the

hydroxy O-atoms of peptide molecules A and B and can be assumed to be involved in H-bonding interactions with these groups.

Finally, **77** was coupled to Z-Leu-OH to give the pentapeptide Z-Leu-Ile-Aib-Pro-Leuol (**78**) in 97% yield, which was deprotected to give quantitatively **73**, *i.e.* the segment 7-11 of HM A3. It is worth to emphasize the surprisingly high yield of the formation of **78**. Then, **73** was coupled with Z-Leu-Aib-Pro-OH (**63**), which was set apart in the synthesis of HM A1 (Chapter 7.2.3). The protected octapeptide Z-Leu-Aib-Pro-Leu-Ile-Aib-Pro-Leuol (**80**) was obtained in 97% yield, which is again a surprisingly high yield for such a coupling reaction. The octapeptide **80** was deprotected to give H-Leu-Aib-Pro-Leu-Ile-Aib-Pro-Leuol (**81**) in 98% yield. Finally, HM A3 was obtained by the successful coupling of **81** with Ac-Aib-Gln-Val-OH (**51**) in 68% yield (*Scheme 20*). The latter had also been prepared in the synthesis of HM A1.

*Scheme 20. Last Coupling Reaction to give HM A3 (**6**)*



Suitable crystals of HM A3 for an X-ray structure determination could be obtained (*Figure 28*). Surprisingly, HM A3 did crystallized in presence of the bacterium *E. Coli* during the biological *in-vitro* assays. It is worth mentioning that no crystals were formed in the analogous solution without *E. Coli*.

The crystals were quite small needles of poor quality and were very weakly diffracting. As a result, there was a paucity of data and the overall quality of the structure was significantly lower than desirable. The overall molecular conformation was clearly defined, but the large standard uncertainties on the geometric parameters mean that no far-reaching conclusions should be drawn from a detailed analysis of individual bond lengths, bond angles or torsion angles. The space group permits the compound in the crystal to be enantiomerically pure, but the absolute configuration of the molecule has not been determined. The enantiomer used in the refinement was based on the known (*S*)-configuration of all peptide units in the molecule. The structure determination confirmed that there were no unexpectedly inverted peptide units present. The asymmetric unit contains one peptide molecule and one disordered water molecule. The peptide molecule displays disorder in several side chains. These are the terminal two methyl groups of Leu^4 and the terminal methyl groups of $Leuol^{11}$, as well as three of the ring C-atoms of Pro^{10}. Most of the amide groups are involved in intramolecular H-bonds, but a few intermolecular interactions exist and serve to link the peptide and H_2O molecules into a two-dimensional network (*Figure 29*).

The hydroxy group forms an intermolecular H-bond with the amide O-atom of the central Pro^6 of a neighboring molecule. This interaction links the peptide molecules into extended chains. The primary amide group of the Gln^2 side chain forms intermolecular H-bonds with two different neighboring peptide molecules, with the

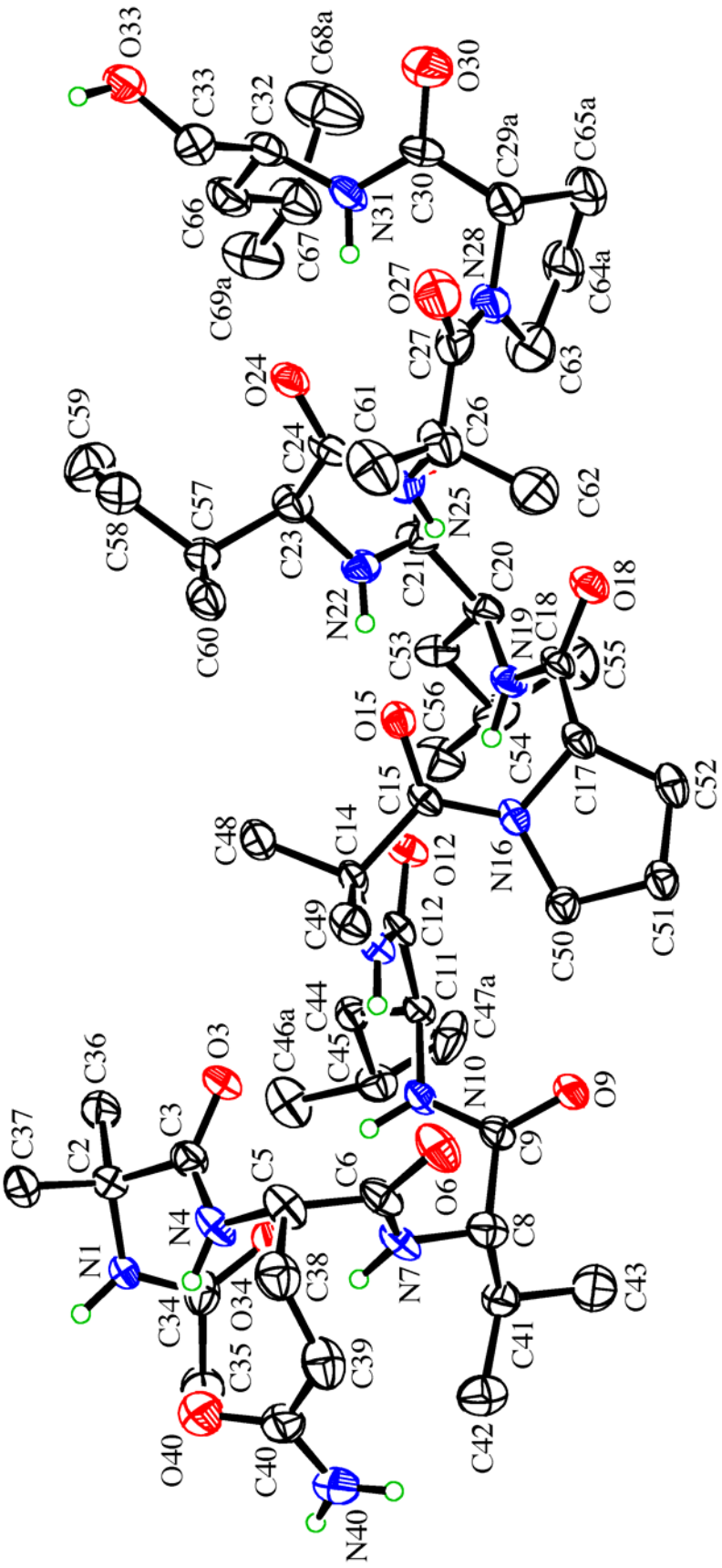


Figure 28. *ORTEP Plot* [86] *of the Molecular Structure of* Hypomurocin A3 (**6**)
(50% probability ellipsoids; H-atoms given arbitrary displacement parameters for clarity)

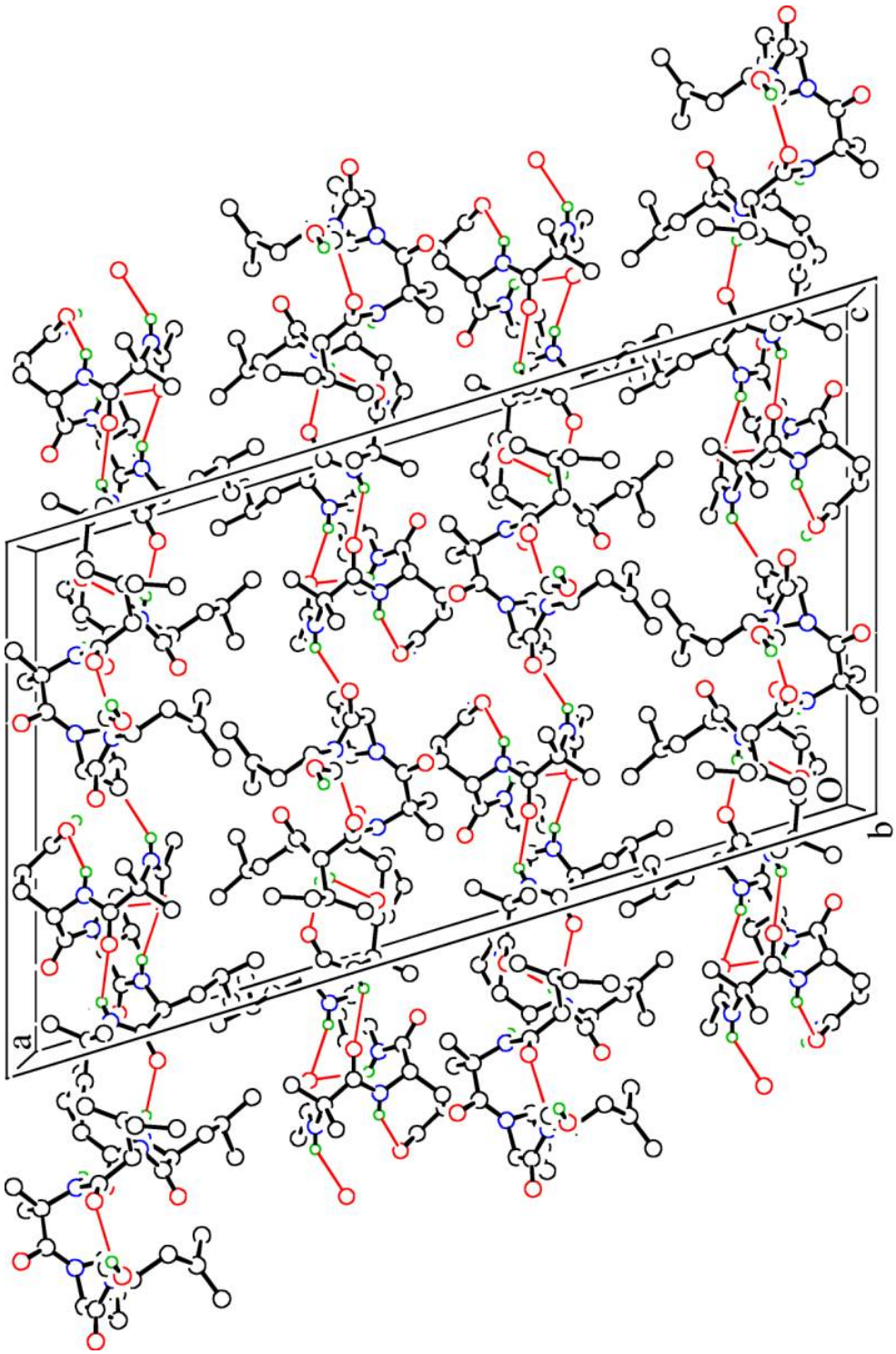


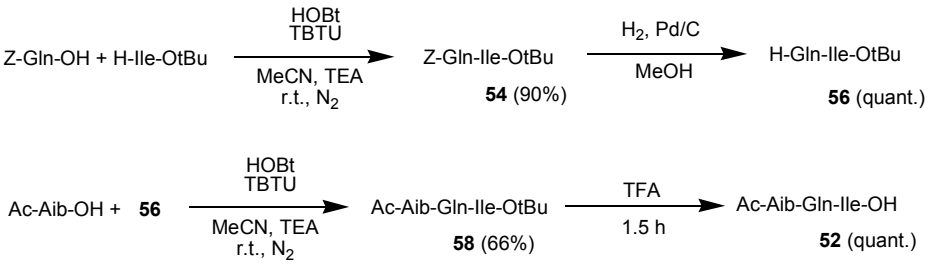
Figure 29. *Molecular Packing of* Hypomurocin A3 *Projected down the* b-*Axis Showing the H-Bonding Scheme* (equivalent isotropic spheres for atoms; uninvolved H-atoms omitted for clarity; color code: C-atoms: black, O-atoms: red, N-atoms: blue, H-atoms: green, and H-bonds: red)

acceptor amide O-atoms being on the Pro^{10} and Aib^{9} moieties. The amide H-atom of Aib^{1} forms an intermolecular H-bond with the O-atom of the disordered H_2O molecule. Although the H-atoms of the H_2O molecule could not be located, suitable O···O distances suggest that the water molecule donates two H-bonds. One interaction is with the hydroxy O-atom in the peptide molecule from which the amide H-atom of Aib^{1} interaction originates, and thereby links pairs of peptide and H_2O molecule into loops. The second interaction is with the amide O-atom of the Gln^{2} side chain of a different peptide molecule, which thereby links pairs of peptide and H_2O molecule into chains. All of the intermolecular H-bonding interactions combine to link the peptide and H_2O molecules into two-dimensional networks. With the exception of the amide H-atom of Aib^{9}, which is not involved in any H-bonding interaction, all amide groups not mentioned above form intramolecular H-bonds with amide O-atoms. The amide H-atoms of Val^{3}, Pro^{6}, Ile^{8}, and $Leuol^{11}$ form intramolecular H-bonds with the amide O-atoms that are seven atoms back along the peptide backbone. The amide H-atoms of Gln^{2} forms an intramolecular H-bond with the amide O-atom of the Gln^{2} side chain, thereby creating a loop. The amide H-atoms of Leu^{4} and Aib^{5} do not interact as expected with the carbonyl O-atoms of Aib^{1} and Gln^{2}, respectively, but interact instead with the carbonyl O-atoms of Ac and Aib^{1}, respectively, to give loops.

7.2.5 *Hypomurocin A5*

The synthesis of HM A5 is rather short in comparison to those of HM A1 and A3. It can be resumed to the synthesis of the segment 1-3 (Ac-Aib-Gln-Ile-OH; **52**). The other part of the synthesis is the coupling of the two segments 4-6 (Z-Ile-Aib-Pro-OH; **75**) from the HM A3 synthesis and 7-11 (H-Leu-Leu-Aib-Pro-Leuol; **67**) from the HM A1 synthesis and, after the deprotection of the segment 4-11, the coupling with **52**. The synthesis of **52** is analogous to that of **51** (*Scheme 21*).

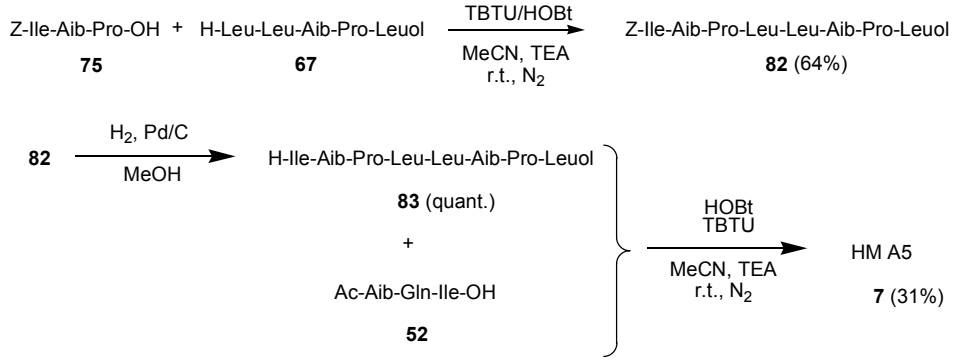
Scheme 21. Synthesis of the Tripeptide **52**



The coupling of Z-Gln-OH with H-Ile-OtBu gave the dipeptide **54** in 90% yield. This compound had the same plaster-like properties as **53** in the synthesis of HM A1 and was also difficult to dissolve in polar solvents. The deprotection of **54** under an H_2-atmosphere (Pd/C) led quantitatively to **56** as a colorless oil. The coupling of Ac-Aib-OH with **56** gave the *tert*-butyl protected tripeptide **58** in 66% yield. The latter was hydrolyzed with TFA in 1.5 h to give **52**, the segment 1-3 of HM A5 (**7**), in quantitative yield.

Finally, the coupling of the three segments 1-3, 4-6 and 7-11 of HM A5 was achieved by using the TBTU/HOBt method (*Scheme 22*).

*Scheme 22. Last Coupling Steps to give HM A5 (**7**)*

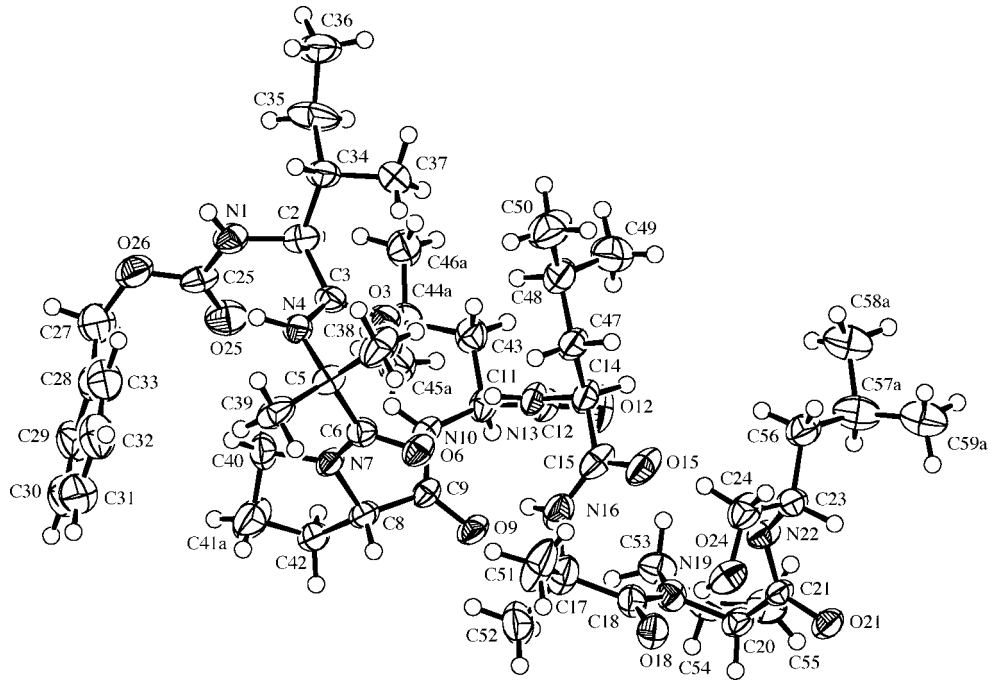


The Z-protected octapeptide **82** was obtained by the condensation of **75** and **67** with TBTU/HOBt in 64% yield. Suitable crystals of **82** for an X-ray crystal structure determination were obtained (*Figure 30*). The deprotection of **82** under the usual conditions gave the octapeptide **83** quantitatively, which was coupled to the previously synthesized tripeptide **52** to give HM A5 in 31% yield.

The space group permits the compound in the crystal to be enantiomerically pure, but the absolute configuration of the molecule has not been determined. The structure determination of **82** confirms that all peptide units do indeed have the *S*-configuration. There are two symmetry-independent molecules in the asymmetric unit. Both have the same stereochemistry although their conformations are slightly different. Some of the side chains in each molecule are disordered over two conformations: the *i*-Pr side chain of Leuol[8] and the entire side chain of Leu[5] of molecules A and B, respectively as well as the *i*-Pr side chain of Leu[4] in molecule A and the entire side chain of Ile[1] in molecule B. In addition, Pro[3] of each molecule has two envelope conformations with the C-atom of the envelope flap being "up" or "down". The asymmetric unit includes two molecules of the peptide plus two disordered solvent molecules, which, from the arrangement of residual electron density peaks, appeared to be AcOEt. The disorder of the solvent molecules could not be modeled adequately. Therefore, the contribution of the solvent molecules to the intensity data was removed by using the SQUEEZE routine [89] of the PLATON program [90].

The hydroxy group in each molecule forms an intramolecular H-bond with the amide O-atom of Pro[7], thereby forming a loop. The amide H-atom of Ile[1] in molecule A forms an intermolecular H-bond with the hydroxy O-atom of a neighboring molecule A. This interaction links the A molecules into extended chains. The corresponding interaction also links the B molecules into extended chains. The amide H-atom of Aib[2] in molecule A forms an intermolecular H-bond with the amide O-atom of Leu[5] of the same neighboring molecule A that accepts the N-H...O interaction with the amide H-atom of Ile[1], so that this interaction is also a link in the chains described above. Molecule B behaves in an identical fashion. The amide H-atoms of Leu[4], Leu[5], Aib[6] and Leuol[8] form intramolecular H-bonds with the amide O-atoms that are seven atoms back along the peptide backbone. Each of these interactions serve to maintain a fairly rigid helical conformation of the peptide. The same pattern of intramolecular interactions occurs in molecule B with one exception. The amide H-atom of Aib[6] in molecule B, does not interact as expected with the carbonyl O-atom of Pro[3] in molecule B, but interacts instead with the carbonyl O-atom of Aib[2] in molecule B to give a loop.

a)



b)

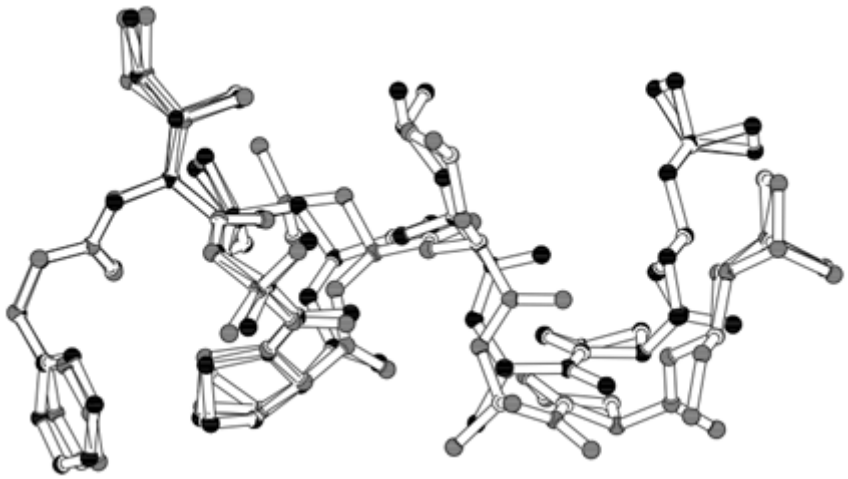


Figure 30. *a) ORTEP Plot* [86] *of the Molecular Structure of* **82** (conformation A, 50% probability ellipsoids; arbitrary numbering of the atoms); *b) Superimposition of the two conformations A and B of the Molecular Structure of* **82**

The N···O and H···O distances for N(Aib^6, molecule B)-H···O(Pro^3, molecule B) are 3.641(6) and 3.00 Å, respectively, and are well beyond the distance considered to be an indication of an H-bonding interaction. Presumably this difference results from a small difference in the turn structure of molecule B compared with that in molecule A. Superposition of the two molecules shows that although the conformations at the Z-end are similar, small deviations develop after the Pro^3 unit.

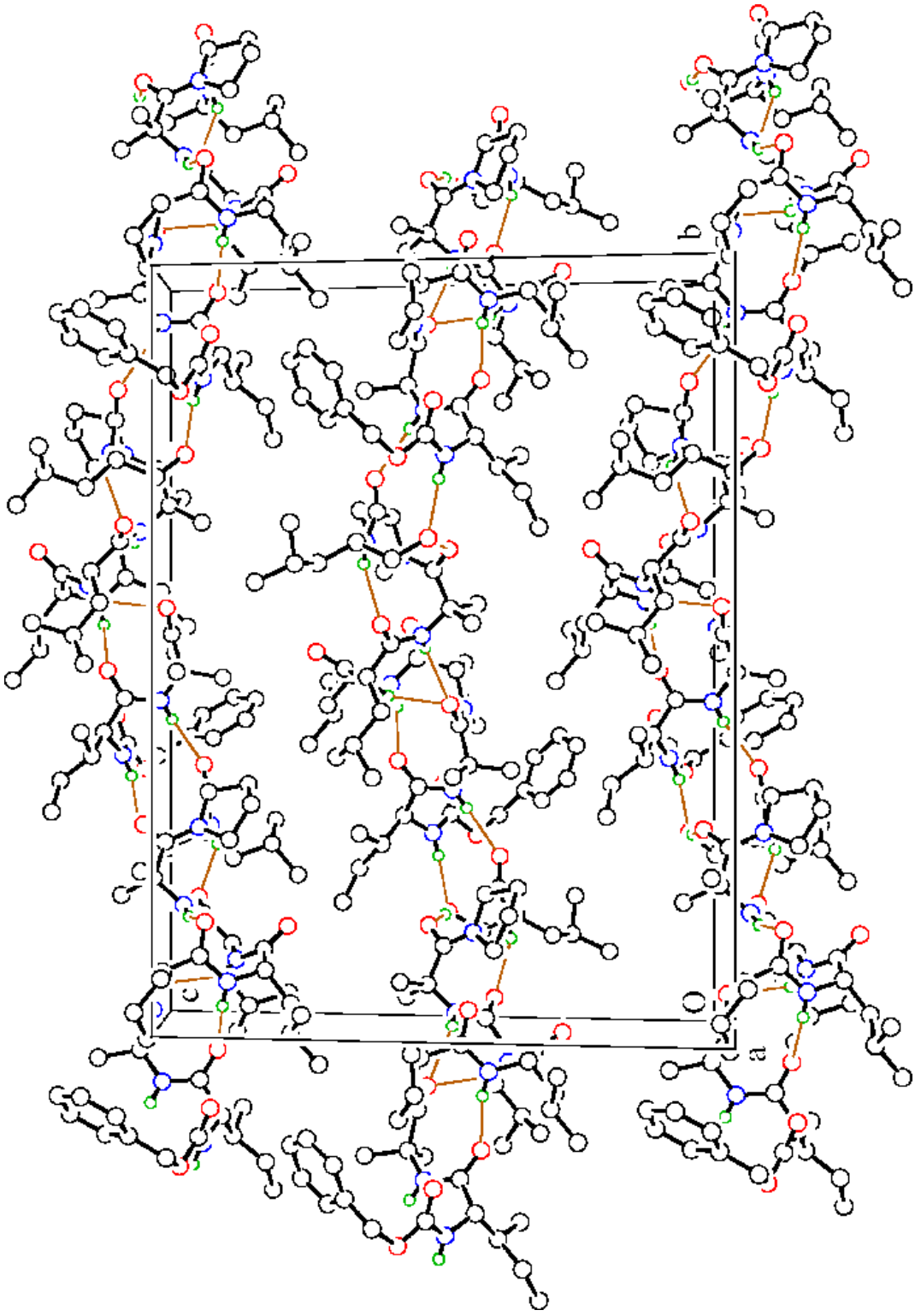


Figure 31. *Molecular Packing of* **82** *Projected down the* a*-Axis Showing the H-Bonding Scheme* (equivalent isotropic spheres for atoms; uninvolved H-atoms omitted for clarity; color code: C-atoms: black, O-atoms: red, N-atoms: blue, H-atoms: green, and H-bonds: red)

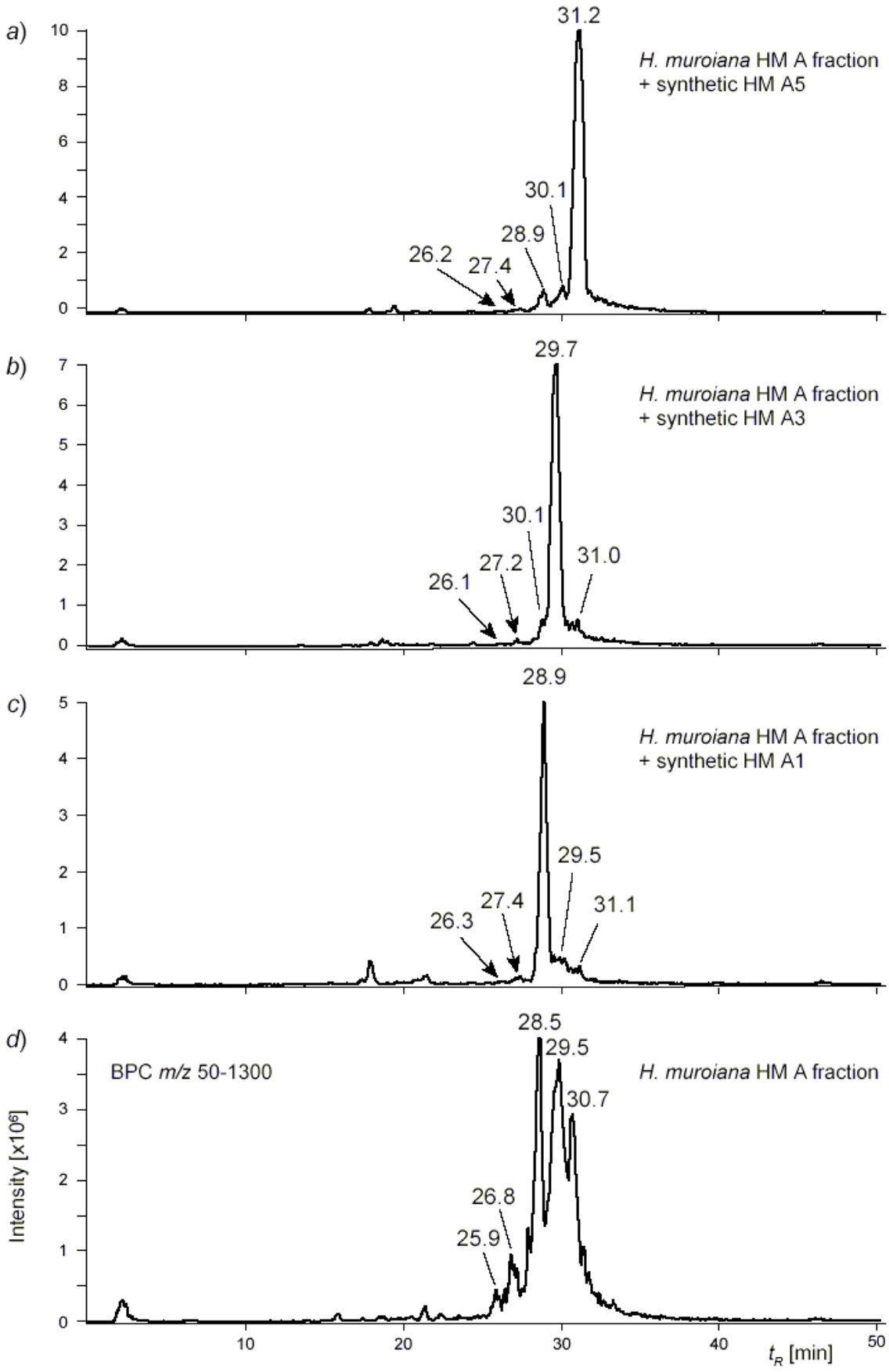
7.2.6 HPLC Correlation between Synthetic and Natural *Hypomurocins*

The synthetic HM A1 (**5**), HM A3 (**6**) and HM A5 (**7**) peptaibols were analyzed individually or as defined mixtures with the fraction containing the set of *Hypomurocins A* (HM A) of the extract of the fungus *H. muroiana* by reversed-phase HPLC-ESI-MS. *Figure 32* shows representatively the base peak chromatogram (BPC) of the natural fungus extract and of mixtures of the natural extract with the three synthetic materials. According to *Brückner* and co-workers, the HM A fraction is a mixture composed of six structurally similar peptaibols possessing three different quasi-molecular ions ($[M + H]^+$) [88]. In our experiment, the natural peptaibol HM A1 ($[M + H]^+$, *m/z* 1161) was recorded at t_R 28.5 min, the three compounds HM A2, A3, and A4 ($[M + H]^+$, *m/z* 1175 each) were all co-eluting at t_R 29.5 min, and the isomeric HM A5 and A5a ($[M + H]^+$, *m/z* 1189 each) were recorded at t_R 30.7 min (*Figure 32d*). It is easily recognised from these chromatograms that the addition of synthetic HM A1 to the natural mixture induces an increase of the peak at t_R 28.9 min (*Figure 32c*). The same experiment performed with synthetic HM A3 produces an increase of the peak at t_R 29.7 min (*Figure 32b*) and the addition of synthetic HM A5 results in an increase of the peak at t_R 31.2 min (*Figure 32a*).

The synthetic peptaibols were further analysed by MS/MS to attest their amino acid sequences (see Experimental Part). Due to the presence of two proline residues in the HM A peptaibols, abundant fragment ions of the *b*- and *y*-type (Nomenclature of the peptide fragment ions according to *Roepstorff* and *Fohlman* [91] and *Biemann* [92]) deriving from cleavages of the amide bond adjacent to the proline N-terminus, are observed in the mass spectra. This gas-phase behaviour of proline-rich peptides has already been reported [93]. In consequence, the intensities of the quasi-molecular ions $[M + H]^+$ observed for the HM A derivatives were low (<1% rel. intens.). The MS/MS data obtained clearly indicated the amino acid sequence of the three synthetic compounds and were identical with the ones recorded for the natural products, thus confirming the structures proposed by *Brückner* and co-workers [88].

(see next page)

Figure 32. *HPLC-ESI-MS Base Peak Chromatograms (BPC) Representing the* Hypomurocin A *(HM A) Fraction of* H. muroiana *Spiked with Synthetic HM A5 (a), HM A3 (b), and HM A1 (c) as well as the BPC of the Natural HM A Sample (d)*


a)
31.2
H. muroiana HM A fraction
+ synthetic HM A5
30.1
28.9
26.2
27.4
b)
29.7
H. muroiana HM A fraction
+ synthetic HM A3
30.1
31.0
27.2
26.1
c)
28.9
H. muroiana HM A fraction
+ synthetic HM A1
29.5
27.4
31.1
26.3
d)
28.5
29.5
BPC m/z 50-1300
H. muroiana HM A fraction
30.7
26.8
25.9
Intensity [x10⁶]
t_R [min]

7.2.7 Conclusion

In conclusion, the three *Hypomurocins A1*, *A3* and *A5* were successfully synthesized. We have shown that the introduction of the two Aib-Pro units *via* the azirine/oxazolone method occurs with almost quantitative yield. The yields were conform or better than expected (see preliminary calculation). The ester hydrolysis with LiOH and deprotection with TFA were also performed in very high yield, as well as the deprotection of the Z group and the coupling of small peptide segments. As expected, the sensitive points in the synthesis were the coupling reactions of the segments: they have been achieved in 41% yield for the final coupling of **51** and **71** and 55% yield for the coupling of **68** and **67** to give the octapeptide **69**. The introduction of the hindered Ac-Aib-OH was accomplished in 63% yield. In the case of HM A3 the yields were excellent, being almost quantitative for five steps. Even the last coupling leading to HM A3 was performed with high yield (68%). For HM A5, yields are also good, except for the final coupling to give HM A5 in only 31% yield as well as for the introduction of Aib^1 with only 66% yield.

An epimerization occured at the Pro^{10} residue in the synthesis during the preparation of the segments 8-11. This was the only epimerization observed in the synthesis of the three *Hypomurocins*. Although the epimer could be separated and isolated, its amount was negligible in term of yields.

These total syntheses illustrate the advantages of the azirine/oxazolone method in the synthesis of peptaibols. Several intermediates or final products were obtained as crystals suitable for X-ray structure determination, which confirmed the helical structure of Aib containing oligopeptides. The final HPLC correlation between synthetic and natural *Hypomurocin* proved to be in accordance, confirming thus the original analytical work on the *Hypomurocins* of *Brückner*.

7.3 Three-Dimensional NMR Investigations

These investigations concern only the *Hypomurocin* HM A1. The investigations for HM A3 and HM A5 will be reported in a coming publication [94].

7.3.1 Three-Dimensional Model in Solution

As expected in the case of peptaibols, the presence of several Aib moieties in the molecule induces a helical conformation despite the presence of Pro moieties, which appear just after the Aib units in the HM A1 sequence.

The structure studies in (D_6)DMSO solution by NMR spectroscopy, *i.e.* the ROESY spectra, showed many characteristic sequential and medium-range NOE connectivities which revealed a well-defined helical conformation of HM A1 with tendencies for the formation of type I(III) β-turns (*Figure 33*).

a)

b)

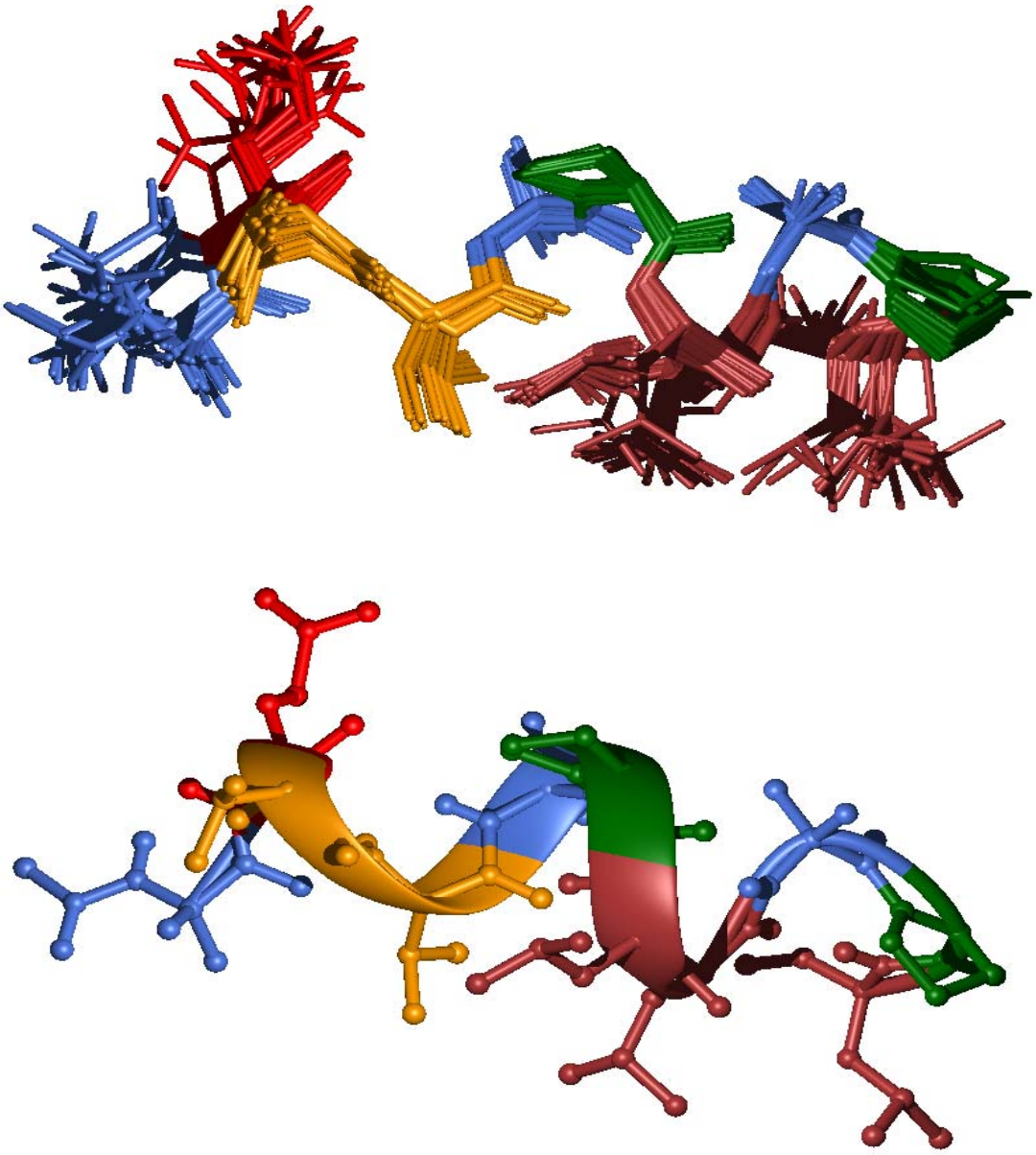


Figure 33. *a) Superposition of the Final 20 NMR Structures for HM A1. The Residues are Color-coded as Following: Aib blue, Gln red, Val orange, Pro green and Leu brown; b) A Typical NMR Structure in Ball-and-Stick Representation. The Secondary Structure is shown in Ribbon Representation*

In particular, the presence of significant sequential NN NOEs, as well as the medium-range αN *(i,i+2), (i,i+3*) and *(i,i+4)* and αβ *(i,i+3*) NOEs were clearly evident for helical and turn-like motives (*Figure 34*). The calculated average structures display a well-defined family of conformation with a RMSD value of the backbone atoms to the mean structure of 0.64 Å, and, excluding the more flexible N- and C-terminal residues 1 and 11, of 0.47 Å.

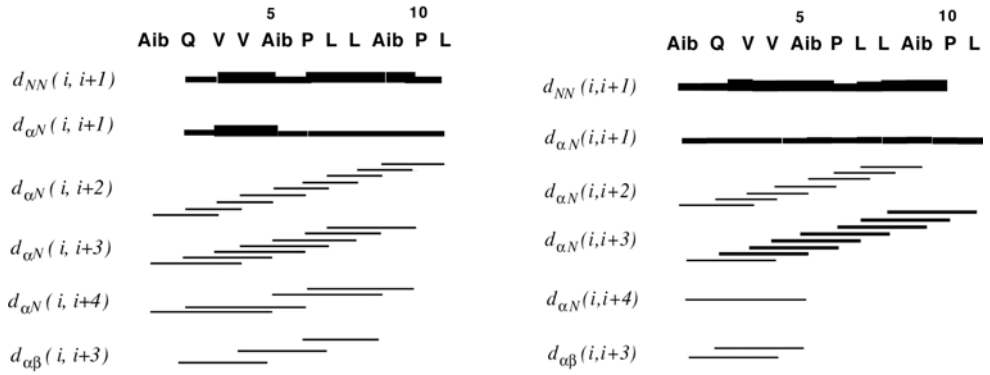


Figure 34. *Summary of Sequential and Medium-Range of NOE Connectivities for HM A1 in DMSO (left) and bound to DPC Micelles (right). NOE Intensities are represented by the Line Thickness (the H^{δ}-Atoms of Pro are considered as Amide H-Atoms, and the H^{β}-Atoms of Aib as H^{α}-Atoms)*

Considering the backbone torsion angles ϕ and ψ determined from the calculated structures, distortions from the ideal ϕ,ψ angles of a right-handed α-helix were observed (*Table 2*).

Table 2. *$^{3}J_{HN\text{-}H\alpha}$ Coupling Constants Derived from the 1D ^{1}H-NMR Spectra, and Calculated ϕ and ψ Angles of the NMR Structures*

Residue	$^{3}J_{HN\text{-}H\alpha}$ (Hz)	ϕ (°)	ψ (°)
Aib[1]	-	-165 ± 86.1	8.3 ± 53.2
Gln[2]	5.4	-50.1 ± 74.3	3.3 ± 5.2
Val[3]	7.1	-91.9 ± 2.2	16.8 ± 0.6
Val[4]	8.5	-108.8 ± 0.2	-16.5 ± 0.4
Aib[5]	-	-41.0 ± 1.8	-63.6 ± 0.6
Pro[6]	-	-62.8 ± 0.8	-25.7 ± 4.7
Leu[7]	7.1	-81.1 ± 6.1	-26.2 ± 5.1
Leu[8]	9.2	-109.3 ± 0.6	22.5 ± 1.2
Aib[9]	-	-39.3 ± 0.2	-54.7 ± 0.3
Pro[10]	-	-67.6 ± 0.2	-1.2 ± 0.2
Leu[11]	8.9	-141.5 ± 8.5	50.6 ± 83.1

In particular, the large $^{3}J_{HN\text{-}H\alpha}$ coupling constants (>8.5 Hz) at residue 4, 8 and 11, resulting in extended torsion angles ϕ, are more likely in β-type structures. In the case of residue 8, a type I β-turn was observed with Leu[7]-Leu[8] at positions *i+1* and *i+2*, respectively.

Finally, HM A1 seems to adopt a stable mixed helical conformation in DMSO-solution with typical structure elements of α-helices, 3_{10}-helices and type I β-turns.

7.3.2 Three-Dimensional Model in Deuterated Micelles

Earlier studies of the structures of peptaibols in solution have been performed mainly in methanol [95]. Later, it was recognized that perdeuterated detergent micelles

display superior properties and led to better-defined structures (see *e.g.* the work of *Anders et al.* describing the structure of *Chrysospermin* [96] or by *Condamine et al.* in the case of *Trichorzianin TA VII* [97]). Since HM A1 represents a membrane-active peptide, the structure of the peptaibol in a membrane-mimicking environment was determined by applying a methodology used earlier (see for example [98]) and described in more detail in [99]. In the present studies, perdeuterated dodecylphosphocholine (DPC) micelles [100] were used in order to mimick biological membranes. The NOESY spectra in DPC micelles revealed rather strong sequential correlations between amide H-atoms. In general, the latter were stronger than the corresponding αN(*i,i+1*) correlations and proved that the backbone fold is predominantly helical and not extended or unfolded. Moreover, some αβ(*i,i+3*) (or, in the case of Aib: QB1/QB2-β(*i,i+3*) and a (non-interrupted) series of αN(*i,i+3*), and αN(*i,i+2*) NOEs was observed (for a summary of the applied restraints see *Figure 34*). The latter are usually observed in 3_{10}-helices but are usually absent for α-helically folded peptides [101]. A superimposition of the output of the structure calculation on the backbone atoms of all residues is displayed in *Figure 35.*

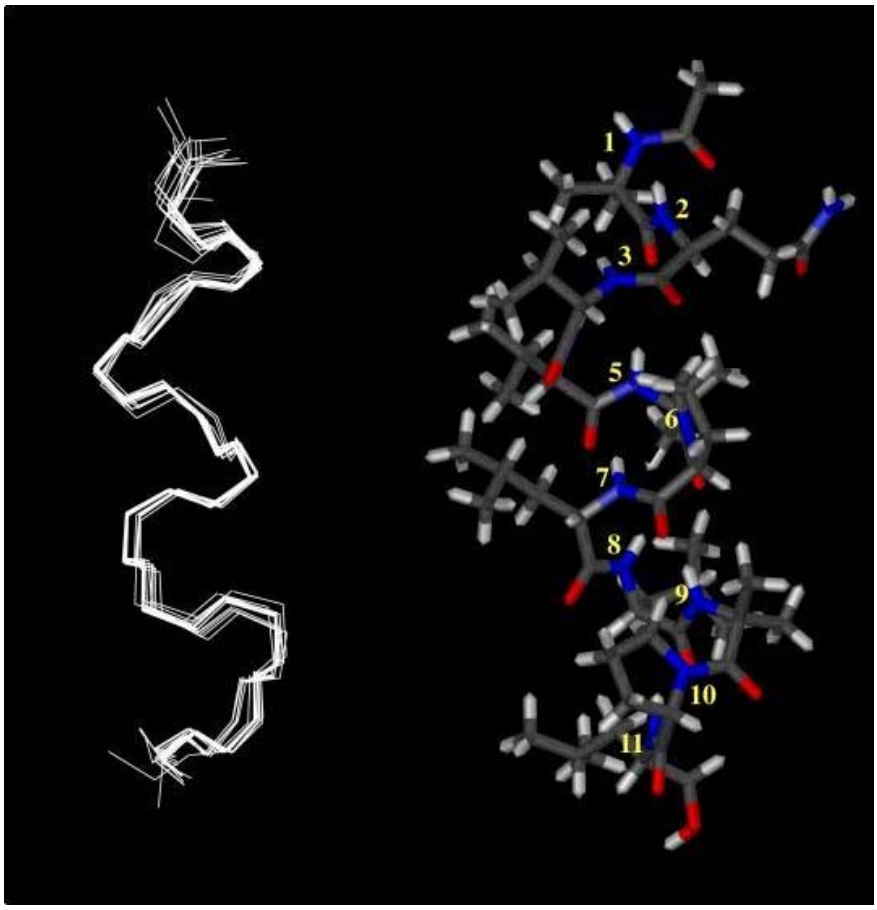


Figure 35. *Left: Backbone Presentation of the Structure of HM A1 in DPC Micelles. Backbone Atoms of all Residues have been used for the Superposition. The N Terminus is at the Top. Right: Full-Atom Presentation of a Representative Conformer of HM A1, in which the Nitrogen-Atoms have been numbered according to their Sequence Position (color code is : N-atoms blue, O-atoms red, H-atoms white, and C-atoms gray. The Orientation of the Molecule is the Same on the Left and Right)*

Although the number of medium-range restraints is rather high, the generated structures cannot be classified according to the *Kabsch-Sanders* algorithm [102] into a unique type of helices. Scalar coupling constants, $^3J_{HN,H\alpha}$ for all residues different from Aib and Pro were also determined. The values are < 4 Hz (Gln^2), 6.0 Hz (Val^3), 5.9 Hz (Val^4), 4.2 Hz (Leu^7) and 6.5 Hz (Leu^8). Although values for stable helices are about 4 Hz, these values are significantly lower than the values for freely rotationally averaged dihedral backbone angles (about 7 Hz) [103]. They also indicate that the backbone is not perfectly rigid. Our failure to derive defined structures partly is attributed to residual flexibility of the peptides, and to the inherently low proton density in such a short peptide. However, the large number of medium-range NOEs shows that the peptide is surprisingly well-defined in a membrane-mimicking environment. The presence of a continuous stretch of $\alpha N(i,i+2)$ NOEs indicates that the structure is predominantly a 3_{10}-helix.

Furthermore, the atom coordinates from the structure of HM A1, as determined by us in a membrane-mimicking environment, were used to compute a space-filling model. Due to the placement of Pro^6 and Pro^{10} at opposite sides of the helix and the small steric requirements of this amino acid, the space-filling representation reveals a S-like shape as in the crystal packing of the octapeptide Z-Val-Aib-Pro-Leu-Leu-Aib-Pro-Leuol (**69**) in the HM A1 synthesis (*Figure 23*).

We notice reasonably good surface complementarities of the front- and backside of the helix, which allows packing of individual peptide molecules against each other, thereby maximizing hydrophobic contacts. It is assumed that the biological function of peptaibols is exhibited by forming channels in membranes. The solution structure of HM A1 (**5**) indicates a way, how individual molecules may form larger aggregates, which result in membrane channels. *Chugh et al.* [38] have constructed a model of a helical bundle of *Trichotoxin A50E* based on the crystal structure of the monomer. In their model, individual helices aligned parallel to each other resulting in an octameric aggregate, in which hydrophilic side-chains exclusively point into the center of the channel. In contrast, the peptaibol HM A1 (**5**) exclusively consists of hydrophobic residues. Moreover, the undecapeptide sequence is too short to form a membrane-spanning helix. We therefore speculate that individual molecules pack against each other as indicated in *Figure 35* (see next page). Two rings, each one resulting from joining a linear array of molecules, may then associate at the termini of the peptides forming a longer tube that is now long enough to pass the membrane.

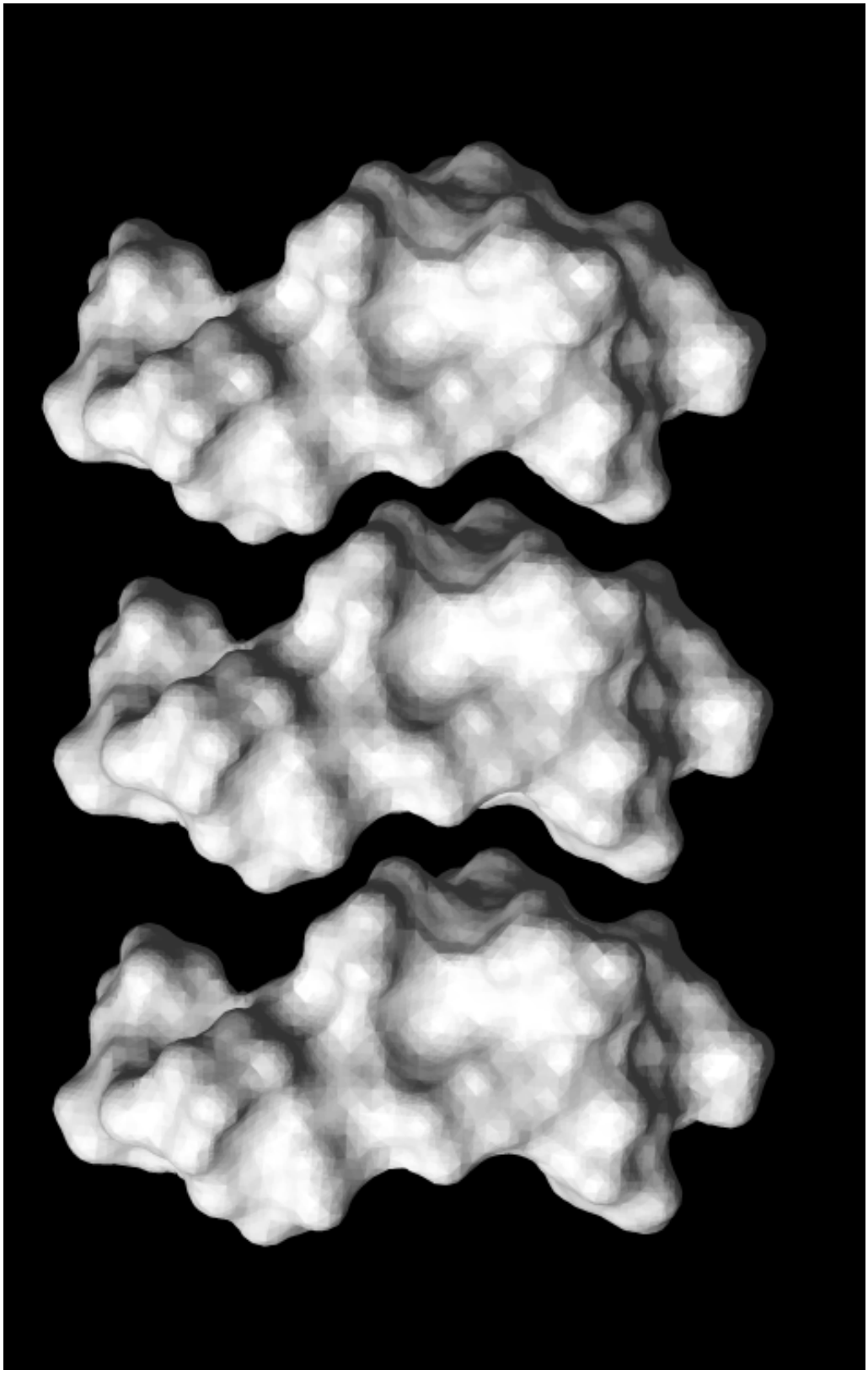


Figure 36. *Space-Filling Model of HM A1 as computed with the Program MOLMOL* [104]. *A Single Conformer has been triplicated and displaced to indicate Possible Modes of Packing. In the Figure the N-Terminus is always at the top.*

7.4 *In-vitro* Biological Investigations

7.4.1 Bacteriological Assays

In order to complete this study, the biological and toxicological assays performed with the three *Hypomurocins* **5**, **6** and **7** are presented here. These compounds are globally pretty similar (*Figure 37*) and do differ only in two or three amino acids. The differences in the side chains are as follows: Ile^3 can be regarded as Val^3 with an ethyl group instead of a methyl group, and Leu^8 is an isomer of Ile^8. Furthermore, Leu^4 can be considered as a "CH_2-extended" Val^4. All three *Hypomurocins* contain only one hydrophilic residue Gln^2, and all others residues are hydrophobic. Thus, a similar reactivity in *in-vitro* assays can be expected.

```
    1   2   3   4   5   6   7   8   9   10  11

Ac-Aib-Gln-Val-Val-Aib-Pro-Leu-Leu-Aib-Pro-Leuol HM A1
Ac-Aib-Gln-Val-Leu-Aib-Pro-Leu-Ile-Aib-Pro-Leuol HM A3
Ac-Aib-Gln-Ile-Ile-Aib-Pro-Leu-Leu-Aib-Pro-Leuol HM A5
```

Figure 37. *Differences in the Structures of HM A1(***5***), A3 (***6***) and A5 (***7***) are in Bold*

The hemolytic activity was tested on blood agar plates prepared with sheep blood on agar medium in *Petri* dishes. The peptaibols were dissolved in a H_2O/MeOH solution. In order to avoid the death of bacteria due to pure MeOH, as much as possible water was added. On the other hand, in order to avoid the precipitation of *Hypomurocins* in water, the minimal volume of MeOH was used. Several concentrations (*Table 3*) for HM A1, A3 and A5 were used and some activity was observed after the plates were left overnight at 37 °C for incubation. The plates were prepared with 20 µl of solution poured over the plates.

Table 3. *Ratio of Solvents in which the* Hypomurocin *is dissolved and Control Solutions*

Peptaibol	HM A1	HM A3	HM A5
Used Amount	4.4 mg	2.3 mg	2.2 mg
Control Solution [a)]	Solution 1	Solution 2	Solution 2
H_2O (%vol.)	60 %	70 %	70 %
MeOH (%vol.)	40 %	30 %	30 %

a) Solution mixture without substance

The plates still contained agar which could react with the substances. Thus, the hemolyses were compared qualitatively to control samples. HM A1 has shown weak β-hemolysis activity only at a concentration of 2.2 mg/ml, HM A3 has shown normal β-hemolysis activity at a concentration of 2.3 mg/ml and weak β-hemolysis at a concentration of 1.15 mg/ml, and HM A5 has shown very weak β-hemolysis activity only at a concentration of 2.2 mg/ml. Lower concentrations did not show any activities. The results are shown in *Table 4*.

Table 4. *Sheep Blood Plates Hemolysis Results for HM A1, A3 and A5*

Substance	μg/ml	Hemolysis	% Methanol	Hemolysis
HM A1	4400	strong	40	weak
	2200	weak	20	no
	1100	no	10	no
HM A3	2300	strong	30	weak
	1150	weak	15	no
	575	no	7.5	no
HM A5	2200	weak	30	weak
	1100	no	15	no
	550	no	7.5	no

The MeOH used to dissolve the peptaibols already occasions the hemolysis of the sheep erythrocytes at the highest used concentrations of 30-40%. Thus, only the results with solutions containing 20% or less methanol can be taken into account. Under these experimental conditions, HM A1 and A3 seem to have a greater hemolysis activity as HM A5.

Another series of assays concerned the microbiological activity. Two Gram positive (*B. Subtilis* and *S. Aureus*) and one Gram negative (*E. Coli*) bacteria strains were used. For *S. Aureus* and *E. Coli*, the occasioned inhibition due to the MeOH in the solvent was too close to the supposed inhibition of the peptaibols. Thus, the experimental values could not be interpreted under these conditions. The minimal inhibition concentrations (MICs) for *B. Subtilis, E. Coli* and *S. Aureus* are shown in *Table 5a*, *b* and *c,* respectively.

Table 5. *MICs for a)* B. Subtilis*; b)* E. Coli*; c)* S. Aureus

a)

Entry	MIC (μg/ml)	Lowest Crystallization Conc. (μg/ml) [a)]	Methanol Control Smallest Inhibitor Conc. (%)
		HM A1	
1	137	137	20
2	137	1100	20
3	68	137	20
4	68	550	20
		HM A3	
5	144	575	7.5
6	72	288	7.5
7	72	144	7.5
		HM A5	
8	550	275	15
9	275	137	7.5
10	275	275	7.5
11	550	68	15

a) Lowest concentration of the solution mixture, where the first crystallization was observed

b)

Entry	MIC (µg/ml)	Smallest Conc. Crystallization (µg/ml)	Methanol Control Smallest Inhibitor Conc. (%)
		HM A1	
12	550	550	5
13	1100	275	20
		HM A3	
14	288	288	3.6
15	1150	1150	15
16	575	288	15
		HM A5	
17	275	275	3.6
18	550	275	7.5
19	1100	137	> 15

c)

Entry	MIC (µg/ml)	Smallest Conc. Crystallization (µg/ml)	Methanol Control Smallest Inhibitor Conc. (%)
		HM A1	
20	>2200	137	20
		HM A3	
21	575	575	15
		HM A5	
22	550	137	7.5

High concentration of MeOH inhibits the growth of *B. Subtilis* and *E. Coli.* Methanol's MIC for *B. Subtilis* and *E. Coli* varies depending on the control solution between a MeOH concentration of 7.5 and 20% for *B. Subtilis* and between 3.6 and 20% for *E. Coli.* This broad range over three dilution steps could be explained by different MeOH contents in the control solutions or by an irregular evaporation of the MeOH while the preparation of the experiment. Therefore, the MICs of the substances must be interpreted carefully if they are close to the inhibition effect of the solvent. This concerns HM A3 and HM A5 for *B. Subtilis* as well as all three substances for *E. Coli.* Eventually, a MIC of 137 µg/ml for the substance HM A1 by *B. Subtilis* could be observed. At higher concentrations, the substances crystallize. This crystal formation reduces the concentration of the dissolved substance. Because a control series without bacteria does not lead to crystals, a certain role played by the bacteria for the crystallization can be assumed. Although the best crystallization was observed with HM A1 in the samples containing *B. Subtilis*, only HM A3 crystals revealed to be suitable for X-ray analysis. The results are summarized in *Table 6*.

Table 6. *Summary of the MICs Results with* B. Subtilis

Peptaibol	Highest Dissolved Amount (μg/ml)	MIC (μg/ml)	Range where the Peptaibol precipitates (μg/ml)
HM A1	2200	68-137	137 - 1100
HM A3	1150	72-144	144 - 575
HM A5	1100	275-550	68 - 275

7.4.2 Virological Assays

The last serie of assays attempted with **5**, **6** and **7** were done with human cells. A first assay concerns the human cells alone being treated with the substances to evaluate the maximal concentration of peptaibol supported by the cells before they are inhibited *i.e*. before they die. Secondly, the human cells were infected by influenza viruses, and the peptaibols were added shortly after in order to test any influence of the peptaibols on the virus infection.

The preparation of the peptaibol solutions is resumed in *Table 7*. Here again, the toxicity of MeOH versus the solubility of the peptaibols in water was a problem. It was decided to use as much MeOH as possible to dissolve the peptaibol and to check this water/MeOH solution alone with the human cells in a control series.

Table 7. *Preparation of the Peptaibol Solutions*

Peptaibol	HM A1	HM A3	HM A5
Used Amount	4.4 mg	2.3 mg	2.2 mg
H_2O	700 μl	600 μl	600 μl
MeOH	300 μl	400 μl	400 μl

- **Assays without virus**

The first attempt was carried out with solutions of the three *Hypomurocins* and the human cells, as prepared in *Table 7*. The mixture was left for 24 h. The results observed are shown in *Table 8*.

Table 8. *Toxicological Results for the* Hypomurocins *on Human Cells (+: living cells; -: dead cells; +/-: part of the cells dead, part of them alive)*

Concentrations [a)]	HM A1	HM A3	HM A5
1:1	-	-	-
1:2	-	-	-
1:4	+/-	-	-
1:8	+	+	+/-
1:16	+	+	+

a) See Table 7. 1:1 = 4.4 mg/ml for HM A1, 2.3 mg/ml for HM A3 and 2.2 mg/ml for HM A5

First, all three *Hypomurocins* have a toxicological effect on the cells, as all cells are dead at concentrations 1:1 and 1:2. The biological activities of the three *Hypomurocins* are not the same, that of HM A5 seems to be much more efficient at lower concentrations. This is quiet surprising if we consider that the three *Hypomurocins* have almost the same structure and three-dimensional space occupation, and assuming that their biological role is a function of the configuration and three-dimensional structure. Finally, for weak concentrations, the *Hypomurocins* are no more active. If the cells are left much longer than one week, they die at all concentrations.

The toxicological limit for the three peptaibols on these human cells are 0.14 mg/ml (1:16) for HM A5 (**7**), 0.29 mg/ml (1:8) for HM A3 (**6**) and 0.55 mg/ml (1:8) for HM A1 (**5**) with the activity scala HM A5 > HM A3 > HM A1.

- **Assays with virus**

The cells status is observed for the first time 12 h after the incubation at 4 °C. The results are shown in *Table 9*. The virus concentration Co for this assay is 0.1 MOI/cell. After 7-8 h, no trace of cell destruction could be detected. Preliminary tests have shown that all cells are dead after 12 h with a virus concentration of 1 MOI/cell.

Table 9. *Cells Status 12 h after Incubation at two Virus Concentrations (+: cells are alive; -: cells are dead)*

Conc.	Co				0.5 Co			
	Control	HM A1	HM A3	HM A5	Control	HM A1	HM A3	HM A5
1:1	-	+	+	+	-	+	+	+
1:2	-	+/-	+/-	+/-	-	+	+	+
1:4	-	+/-	+/-	+/-	-	+/-	+/-	+/-
1:8	-	-	-	-	-	-	-	-
1:64	-	-	-	-	-	-	-	-

For concentrations 1:4 to 1:64, the human cells were almost dead or all dead, which shows that the virus had completely destroyed them. This means that the low concentrations of peptaibols have had no effect on the activity of the virus, as the concentration of peptaibols is too low to harm the human cells, this result relies only on the virus effect.

For concentrations 1:1 and 1:2, the human cells are still alive and have been very little destroyed or not at all destroyed by the virus. This means that the peptaibols interact with the virus in such a way that both the virus and the peptaibols do not react with the cells but rather together first. This might be because the peptaibols interact strongly with the virus membranes, so that through ion channel opening the viruses are destroyed or inhibited and can not infect the human cells.

The cells status is observed again after 18 h and 22 h, but no significant difference was noticed in comparison with the effects observed after 12 h. At the concentrations 1:2 and 1:4, the virus effect seemed to be slowed.

7.4.3 Conclusions from the Biological Tests

As expected in accordance to their length, the biological activity of the *Hypomurocins A1*, *A3* and *A5* was weak but not negligible. Short peptaibols are known to have a weaker activity than long peptaibols.

The peptaibols *Hypomurocin A1*, *A3* and *A5* have small effects on sheep blood agar plates, against *B. Subtilis*, on human cells and they slow down or inhibit the action of the influenza virus on human cells. From all three *Hypomurocins*, HM A3 is the most active on blood agar plates, whereas HM A5 is most efficient on human cells.

Although these peptaibols differ only in their structures and have the same global three-dimensional conformation, their biological activity is not equal.

7.5 Conclusion

The first total synthesis of *Hypomurocins A1*, *A3* and *A5* in solution phase has been successfully achieved by using the azirine/oxazolone method for the introduction of the Aib-Pro sequences included in this undecapeptaibols in one step by using methyl 2,2-dimethyl-2*H*-azirine-3-prolinate as the building block. The coupling reactions of the Z-protected amino acids or peptide acids by using TBTU and HOBt led to the peptides in good to very good yields. The peptides were purified by reverse-phase HPLC (RP-HPLC) and characterized by NMR spectroscopy (^{1}H, ^{13}C, COSY, TOCSY, HSQC, HMBC, ROESY), ESI-MS, IR spectroscopy, elemental analysis, optical rotation, and X-ray crystallography. It was shown by RP-HPLC comparison that the synthetic peptaibols were identical with the natural peptaibols.

A three-dimensional NMR analysis of HM A1 was carried out in DMSO and in deuterated micelles to perform a structural comparison of the helix in solution and in membranes. In all of these three-dimensional investigation, the typical helical conformation was observed. Moreover, the usual S-like shape conformation observed in the self-assembly of peptaibols to form ion channels has also been detected in the presented structures.

Biological investigations have shown that the three peptaibols show some biological activity. Experiments on blood agar plates, bacteria, viruses and human cells illustrated the activity of the three peptaibols. But as known in the literature, as their length do not exceed eleven residues, these effects are weak.

Despite their structural differences due to their side chains, the *Hypomurocins A1*, *A3* and *A5* have shown high similarities in their conformation in solution, crystals and micelles. Moreover, some differences were also detected in the biological activity of HM A1, A3 and A5, HM A3 being the most active molecule in bacteriological tests, and HM A5 being the most active one in the virological tests.

8 General Conclusion and Outlook

The solution-phase synthesis of the protected 6-16 segment of *Zervamicin II-2* and the solution-phase total synthesis of the *Hypomurocins A1, A3* and *A5* were successfully performed. The use of 2*H*-azirin-3-amines for the introduction of highly hindered amino acids or dipeptide units such as Aib, Aib-Pro and Aib-Hyp into peptide chains, which is referred to as the 'azirine/oxazolone method', was carried out with good to very good yields. This method has been prooved as the method of choice for its easiness and efficiency.

Structural investigations by different methods of the *Hypomurocins A1*, *A3* and *A5* have shown the typical helical conformation, in accordance with the high proportion of Aib moities in the molecules. This structural study is not fully completed and needs to be consigned for HM A3 and A5. The detailed comparison of the structures of the three *Hypomurocins* is an attractive task.

Biological investigations of the synthesized *Hypomurocins* was achieved as an illustration of the property of peptaibols to form ion channels in membranes. As expected in regard to the length of these peptaibols, the biological activity was weak.

As an outlook, the following aspects may be investigated in future:

- X-ray crystal-structure determination of HM A1 and A5 and their comparison with HM A3
- Synthesis of one or several peptaibols of the *Hypomurocin B* family
- Investigation of their biological activities as performed with HM A1, A3 and A5
- Comparison of different properties (structure, activity…) of the *Hypomurocin* and other SF4-family peptaibols
- Conductance analyses and determination of the *n*-mer ion channel model for HM A1, A3 and A5 as a completion of this topic and as an illustration of this *n* value for small peptaibols

9 Experimental Part

9.1 General Remarks

9.1.1 Abbreviations

The amino acid and peptide nomenclature conforms to IUPAC-IUB rules (*J. Peptide Sci.*, **2003**, *9*, 1).

Å	Angström
Ac	acetyl
Aib	α-aminoisobutyric acid
aq.	aqueous
BnO	benzyloxy
calc.	calculated
CAMHB	cation adjusted *Mueller-Hinton* bouillon
COSY	*CO*rrelation *S*pectroscop*Y*
DABCO	1,4-diazabicyclo[2.2.2]octane
DMF	dimethylformamide
DMSO	dimethylsulfoxide
equiv.	equivalent
ESI-MS	Electrospray Ionization Mass Spectrometry
g	gram
GCMS	Gas Chromatography/Mass Spectrometry
HETCOR	*HET*eronuclear chemical shift *COR*relation
HMBC	*H*eteronuclear *M*ultiple *B*ond *C*orrelation
HOAt	1-hydroxy-7-azabenzotriazole
HOBt	1-hydroxybenzotriazole hydrate
HSQC	*H*eteronuclear *S*ingle-*Q*uantum *C*orrelation
h.v.	high vacuum
Hyp	L-*trans*-4-hydroxyproline
i-Pr	isopropyl
IR	infrared
Iva	isovaline
l	liter
Leuol	L-leucinol ((*S*)-2-amino-4-methyl-1-pentanol)
Me	methyl
MHB	*Mueller-Hinton* bouillon
MIC	minimal inhibition concentration
min	minute(s)
MOI	Multiplicity Of Infection. Ratio of infectious virus particles to cells
M.p.	melting point
nm	nanometers
NMR	nuclear magnetic resonance
Pheol	L-phenylalaninol ((*S*)-2-amino-3-phenyl-1-propanol)

PLC	preparative layer chromatography
prep.	preparative
Pro	L-proline
ROESY	*R*otating-frame *O*verhauser *E*nhancement *S*pectroscop*Y*
RP-HPLC	Reverse-Phase High Pressure Liquid Chromatography
r.t.	room temperature
SPS	solution-phase synthesis
SPPS	solid-phase peptide synthesis
*t*Bu	*tert*-butyl
TBTU	*O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate
TEA	triethylamine
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	tetramethylsilane
TOCSY	*TO*tal *C*orrelation *S*pectroscop*Y*
Valol	L-valinol ((*S*)-2-amino-3-methyl-1-butanol)
Z	benzyloxycarbonyl

9.1.2 Material and Methods

Starting materials, reagents and solvents

The starting materials 2,2-dimethyl-2*H*-azirin-3-amine (**1**), methyl *N*-(2,2-dimethyl-2*H*-azirin-3-yl)-L-prolinate (**2**), (2*S*,4*R*)-4-(benzyloxy)-*N*-(2,2-dimethyl-2*H*-azirin-3-yl)-L-prolinate (**3**) and Ac-Aib-OH were prepared according to standard procedures [2], [51], [53] and [81], respectively.

Amino acids and the amino alcohols Pheol and Leuol were purchased by *Novabiochem* and *Bachem*, and are all L-configured.

Reagents and solvents were purchased from *Acros*, *Fluka* and *Merck*. All solvents were purified by standard methods, and purchased chemicals where used without further purification.

Chromatographic methods

Thin layer chromatography (TLC): on *Merck* TLC aluminium sheets, silica gel *60* F_{254}. Solvents systems used for the TLCs were AcOEt (A), AcOEt/MeOH 6:1 (B), and AcOEt/MeOH 3:1 (C). The chromatograms were visualised with *Schlittler* reagent (1 g of H_2PtCl_6 in 6 ml of H_2O, addition of 20 ml of 1N HCl, followed by 22.5 g of KI dissolved in 225 ml of H_2O, and H_2O to complete to 1 l).

Preparative layer chromatography (PLC): on *Merck* PLC plates (glass), silica gel *60* F_{254}, 40-63 μm, 2 mm.

Flash column chromatography using *Merck* silica gel *60*, 40-63 μm.

Melting points

M.p.s were measured on a *Büchi B-540* apparatus, uncorrected.

Optical rotations

Optical rotations were measured on an *Perkin-Elmer 241 MC* polarimeter.

Spectroscopic methods

Infrared (IR) *spectra*: IR spectra were recorded on a *Perkin-Elmer Spectrum One* spectrometer, in KBr, absorptions in cm^{-1}.

Nuclear magnetic resonance (NMR) *spectra*: ^{1}H- and ^{13}C-NMR spectra were recorded at 300 K on a *Bruker ARX-300* spectrometer at 300 and 75.5 MHz, resp., employing CD_3OD as solvent as well as internal standard or $CDCl_3$ as solvent and TMS as internal standard. For structural investigations of HM A1, A3 and A5, additional spectra were recorded in d_6-DMSO. Chemical shifts δ are given in ppm and coupling constants *J* in Hz. COSY spectra were recorded either on a *Bruker ARX-300* or *Bruker DRX-600* spectrometer. TOCSY, HSQC, HMBC and ROESY spectra were recorded on a *Bruker DRX-600* spectrometer. All spectra were exploited with the NMR data-processing software *Mest-ReC* [105].

Electrospray mass spectrometry (ESI-MS): ESI-MS were measured on a *Finnigan TSQ-700* triple-stage quadrupole instrument. The compounds were dissolved in MeOH (HPLC grade) or in a 0.1% NaI solution in MeOH (c ≈ 5nmol/ml). ESI operating conditions (positive mode): 16 scans average, capillary voltage 4500V, heated capillary temperature 210 °C, N_2 (30 PSI).

Elemental analysis

Elemental analysis were run on an *Elemental Vario EL* apparatus (C,H,N,S).

RP-HPLC systems (purification)

Analytic (I): Apparatus: *Varian 2550 Variable l Detector* and *2510 HPLC Pump*. Column: *CC 250/4.6 Nucleosil 100-7 C8*, *Macherey-Nagel*. Conditions: Flow rate 1 ml/min, reponse standard, mode normal, lamp D2, range 0.08.

Preparative (II): Apparatus: *Jasco UV-975 UV/Vis Detector* and *PU-987 Pump*. Column: *VP 250/21 Nucleosil 100-7 C8*, *Macherey-Nagel*. Conditions: Flow rate 4 ml/min, reponse standard, mode normal, lamp D2, range 0.08. Solvent: MeCN/MeOH/H_2O 4/3/3 (v/v/v). Retention times t_R in min and wave lengths λ in nm.

RP-HPLC systems (correlation of synthetic and natural *Hypomurocins)*

HPLC-MS: *Hewlett-Packard HP 1100 HPLC* system (*Hewlett-Packard*, Palo Alto, CA, U.S.A.) equipped with a *HTS PAL* autosampler (*CTC Analytics*, Zwingen, Switzerland), connected to a *Bruker ESQUIRE-LC* quadrupole ion trap instrument (*Bruker Daltonik GmbH,* Bremen, Germany) equipped with a *Hewlett-Packard* Electrospray (ESI) ion source (*Hewlett-Packard Co.*, Palo Alto, CA, U.S.A.).

Solvents and reagents: MeCN (HPLC grade, *Scharlau*, Barcelona, Spain), HCOOH *puriss. p.a.* (*Fluka*, Buchs, Switzerland). H_2O was purified with a *MILLI-*Q_{RG} apparatus (*Millipore*, Milford, MA, U.S.A).

Samples preparation: Synthetic material (40 μg) was dissolved in a solution of H_2O/MeCN 9:1 (500 μl), and the fraction containing the natural HM A peptaibols was dissolved in a solution of H_2O/MeCN 9:1 (70 μl). Samples of 1 μl of the synthetic peptaibol stock solution, of 1 μl of the natural HM A peptaibols stock solution, or of a 1:1 mixture of synthetic and natural stock solution were injected and analyzed by HPLC-ESI-MS.

Column and chromatographic conditions: *Interchrom Uptisphere* C_{18} *HDO* column (UP3HDOA5, 3 μm, 0.25×50 mm, *Interchim*, Montluçon, France), the eluent from the HPLC was splitted using an *Acurate* high pressure pre-column flow splitter (*LC Packings*, Amsterdam, The Netherlands) and the output flow was set at a rate of 3 μl min^{-1}. Mobile phase: gradient over 50 min from 10 to 90% of solvent *B* (solvent *A*: 0.1% solution of HCOOH in H_2O, solvent *B*: 0.1% solution of HCOOH in MeCN).

MS conditions: Nebulizer gas (N_2) 13 psi, dry gas (N_2) 8 l min^{-1}, dry temperature 280°, capillary voltage 4500 V, end plate –500 V, capillary exit 70 V, and skimmer 1 19 V. The MS acquisitions were performed at normal resolution (0.6 u full width at half maximum), under ion charge control (ICC) conditions (10'000) in the mass range from *m/z* 50 to 1300 and an *m/z* 50.2 trap drive value. The fragmentation experiments were performed in the auto MS/MS mode by selecting 2 precursor ions, the isolation width was 4 u, the fragmentation cut-off set by "fast calc", and the fragmentation amplitude set at 0.7 V in the "SmartFrag" mode.

X-ray crystal structures

Reports of the crystal structures of the following compounds are available with *PD Dr. A. Linden* at the University of Zürich under the corresponding references:
6: HG 0534; **33**: HG 0301; **38**: HG 0422; **47**: HG 0531; **60**: HG 0424;
68: HG 0430; **69**: HG 0458; **72**: HG 0442; **76**: HG 0426; **77**: HG 0529;
79: HG 0455; **82**: HG 0459; **84**: HG 0352; **85**: HG 0530;

Three-dimensional solution structure calculations

One- and two-dimensional ^{1}H-NMR spectra were recorded at 600 MHz (*Bruker DRX-600* spectrometer), typically at a peptide concentration of 10-20 mg/ml in d_6-DMSO at

300 K. Distance restraints for the data derived from spectra recorded in DMSO or methanol were obtained from ROESY spectra with a mixing time of 250 ms. Spectra were typically collected with 1024 x 256 complex data points zero-filled prior to *Fourier* transformation to 2048 x 1024, and transformed with a cosine-bell weighting function. Data processing was carried out with XWIN-NMR (*Bruker*) and XEASY [106]. The ROESY cross-peak intensities were corrected using the intensity-ratio method in which correction factors due to the spin-lock pulse offset were included [107]. Additionally, data were recorded in 90%H_2O/10%D_2O, 300mM d_{38}-DPC, 20mM MES buffer at 2mM peptide concentration, pH 6.0. Therein, spectra were assigned by using a combination of 12 and 40 ms clean-TOCSY spectra [108] as well as an 120 ms NOESY [109], [110] largely by following the sequential resonance assignment methodology developed by *Wüthrich* and co-workers [101]. All structure calculations were performed applying the simulated annealing protocol implemented in the program DYANA [111], which is based on restrained molecular dynamics in torsion angle space. Starting from 100 randomised conformations, a bundle of 20 conformations, which have the lowest DYANA target energy function, was selected. The program MOLMOL [104] was used for structure analysis and visualisation of the molecular models.

Biological assays

- Bacteriological assays

Bacteria strains:

B. Subtilis	*n.a.*
S. Aureus	ATCC25923
E. Coli	ATCC25922

Method: Miniatur dilution test for the determination of MICs of antibiotica, according to NCCLS guidelines

Bouillon solution: MHB or CAMHB (cation adjusted *Mueller-Hinton* bouillon), prepared according guidelines NCCLS 32.3025/1 and 32.3009/1

- Virological assays

Virus strain:

Virus:	Influenza A virus (FPV-B 293-T)
Designation:	FLUA 183pA
Family:	Orthomyxoviridae
Strain:	Influenza A/FPV/Bratislava
Isolate:	Bratislava
Serotype:	H7N1

Human cells strain:

Human Embryonal Kidney Fibroblast Cells HEK 283

9.2 Protocols

Protocol A: Peptide Coupling

To a solution of an N-protected amino acid or N-protected peptide and 1 equiv. of a C-protected amino acid or C-protected peptide in MeCN were added 1 equiv. of TBTU and HOBt (12% aq.). When the mixture was completely dissolved, 3 equiv. of TEA were added. The reaction was stirred overnight at room temperature (N_2 atmosphere) and checked by TLC. After completion of the reaction, the mixture was washed three times with brine. The organic layer was evaporated and the product was purified by flash chromatography, or PLC.

Protocol B: Coupling with 2*H*-Azirin-3-amines

To a solution of 1.2 equiv. of the corresponding 2*H*-azirin-3-amine in THF or CH_2Cl_2, 1 equiv. of an N-protected amino acid or N-protected peptide was added, and the mixture was stirred at room temperature overnight (N_2 atmosphere). After completion of the reaction, the solvent was evaporated and the product was purified by flash chromatography, or PLC.

Protocol C: Saponification of Peptide Methyl Esters

To a solution of the peptide ester in a mixture of THF, MeOH, and H_2O (3:1:1), 3 equiv. of $LiOH \cdot H_2O$ were added, and the mixture was stirred at room temperature overnight (N_2 atmosphere). After completion of the reaction (checked by TLC), the mixture was acidified with 1N HCl to reach pH 1 and then extracted three times with CH_2Cl_2, the organic layers were combined, dried on Na_2SO_4 and evaporated. The product was purified by flash chromatography, or PLC.

Protocol D: Hydrogenolytic Deprotection

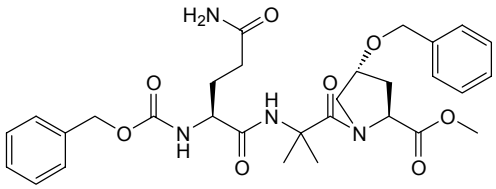
The N-protected peptide was dissolved in MeOH, and 5% Pd/C (1/3 of the weight of the peptide) was added to the solution. The mixture was stirred overnight under H_2 atmosphere at room temperature. After completion of the reaction (checked by TLC), the solution was filtrated through a *celite* path and the solvent was evaporated. The product was dried in high vacuum and used for the next reaction step without any further purification.

Protocol E: Acidic Hydrolysis of Peptide Amides

To a solution of the peptide amide in THF, the same volume of 6N HCl was added at 0 °C and the reaction was stirred overnight at room temperature (N_2 atmosphere). After completion of the reaction (checked by TLC), the solution was extracted with CH_2Cl_2. The organic layers were combined and dried over $MgSO_4$. After evaporation of the solvent, the product was dried in high vacuum and used for the next reaction step without any further purification.

9.3 Experiments

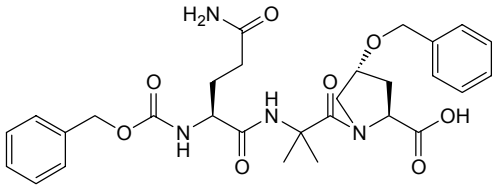
Synthesis of Z-Gln-Aib-Hyp(OBn)-OMe (44)



According to protocol B, the reaction was carried out with 50 mg (0.178 mmol) of Z-Gln-OH and 57.3 mg (0.190 mmol) of **3** dissolved in 3 ml of dry THF. After 48 h, the mixture was purified by flash chromatography (solvents systems (B) to (C)) and the product was dried in high vacuum to obtain 81.5 mg (78.7%) of **44** as a slightly yellowish foam. R_f(B) = 0.05, R_f(C) = 0.65. M.p. 75-78 °C.

IR (KBr): 698*s*, 740*s*, 1028*s*, 1053*s*, 1083*s*, 1178*s*, 1207*s*, 1244*s*, 1363*s*, 1414*s*, 1454*s*, 1531*s*, 1625*vs*, 1667*vs*, 1720*vs*, 2950*m*, 2988*m*, 3033*m*, 3063*m*, 3308*s*.
^{1}H-NMR (300 MHz, CD_3OD): 1.45 (*br. s*, 2 βCH_3^{Aib}), 1.78-2.12 (*m*, βCH_2^{Gln}, βCH_{2a}^{Hyp}), 2.12-2.25 (*m*, βCH_{2b}^{Hyp}), 2.30 (*t*, *J* = 7.5 , γCH_2^{Gln}), 3.68 (*s*, OMe), 3.70-3.87 (*m*, δCH_2^{Hyp}), 4.17-4.27 (*m*, αCH^{Gln}, γCH^{Hyp}), 4.43-4.55 (*m*, αCH^{Hyp}, CH_2^{OBn}), 4.92, 5.00 (*AB*, *J* = 12.6, CH_2^Z), 7.20-7.36 (*m*, 10 arom. CH).
^{13}C-NMR (75.5 MHz, CD_3OD): 24.7, 25.4 (2 *q,* 2 βCH_3^{Aib}), 29.1 (*t,* βCH_2^{Gln}), 32.3 (*t,* γCH_2^{Gln}), 34.0 (*t,* βCH_2^{Hyp}), 52.5 (*q,* OMe), 53.7 (*d,* αCH^{Gln}), 55.5 (*s,* αC^{Aib}), 57.4 (*t,* δCH_2^{Hyp}), 60.9 (*d,* αCH^{Hyp}), 67.7 (*t,* CH_2^Z), 72.1 (*t,* CH_2^{OBn}), 78.3 (*d,* γCH^{Hyp}), 128.7, 128.8, 129.0, 129.4 (4 *d,* 10 arom. CH), 138.0, 139.4 (2 *s,* 2 arom. C), 158.2 (*s,* $OCONH^Z$), 173.0, 174.3, 174.4, 177.6 (4 *s,* 4 CO).
ESI-MS (MeOH+NaI): 621.2 (40%, $[M+K]^+$), 605.3 (100%, $[M+Na]^+$), 583.3 (45%, $[M+H]^+$).

Synthesis of Z-Gln-Aib-Hyp(OBn)-OH (30)

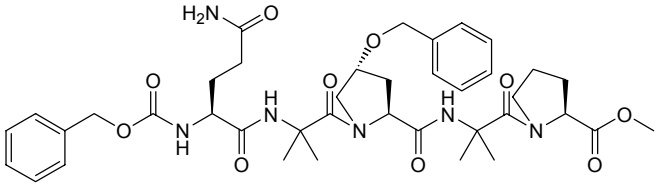


According to protocol C, the reaction was carried out with 893.4 mg (1.533 mmol) of **44** and 193.2 mg (4.600 mmol) of $LiOH \cdot H_2O$ dissolved in 56 ml of a mixture of THF, MeOH, and H_2O (3:1:1). The mixture was stirred overnight, purified by flash chromatography (solvents systems (B) to (C)), and the product was dried in high vacuum to give 776 mg (89%) of **30** as a colorless foam. R_f(C) = 0.05. M.p. 110-114 °C.

IR (KBr): 640*m*, 698*s*, 739*s*, 1028*s*, 1054*s*, 1083*s*, 1178*s*, 1183*s*, 1248*s*, 1334*s*, 1366*s*, 1422*s*, 1454*s*, 1530*s*, 1621*vs*, 1667*vs*, 1712*vs*, 2940*m*, 2987*m*, 3033*m*, 3063*m*, 3324*s*.

^{1}H-NMR (300 MHz, CD_3OD): 1.46 (*br. s*, 2 βCH_3^{Aib}), 1.81-2.13 (*m*, βCH_2^{Gln}, βCH_{2a}^{Hyp}), 2.14-2.27 (*m*, βCH_{2b}^{Hyp}), 2.31 (*t*, *J* = 7.5, γCH_2^{Gln}), 3.68-3.83 (*m*, δCH_2^{Hyp}), 4.16-4.26 (*m*, αCH^{Gln}, γCH^{Hyp}), 4.42-4.55 (*m*, αCH^{Hyp}, CH_2^{OBn}), 4.94, 5.01 (*AB*, *J* = 12.7, CH_2^{Z}), 7.20-7.36 (*m*, 10 arom. CH).
^{13}C-NMR (75.5 MHz, CD_3OD): 24.8, 25.2 (2 *q,* 2 βCH_3^{Aib}), 29.1 (*t,* βCH_2^{Gln}), 32.3 (*t,* γCH_2^{Gln}), 34.4 (*t,* βCH_2^{Hyp}), 53.9 (*d,* αCH^{Gln}), 55.6 (*s,* αC^{Aib}), 57.6 (*t,* δCH_2^{Hyp}), 61.4 (*d,* αCH^{Hyp}), 67.7 (*t,* CH_2^{Z}), 72.0 (*t,* CH_2^{OBn}), 78.3 (*d,* γCH^{Hyp}), 128.7, 128.8, 129.0, 129.4 (4 *d,* 10 arom. CH), 139.4, 138.0 (2 *s,* 2 arom. C), 158.2 (*s,* OCONH^{Z}), 173.2, 174.1, 176.4, 177.7 (4 *s,* 4 CO).
ESI-MS (MeOH+NaI): 567.3 (100%, [*M*-H]$^+$).

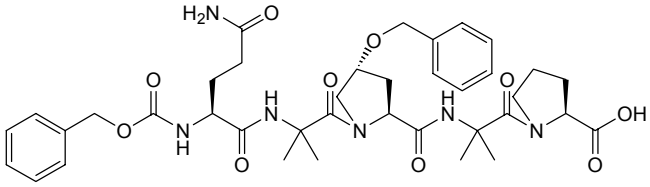
Synthesis of Z-Gln-Aib-Hyp(OBn)-Aib-Pro-OMe (45)



According to protocol B, the reaction was carried out with 170.9 mg (0.300 mmol) of **30** and 64.7 mg (0.330 mmol) of **2** dissolved in 10 ml of dry THF. Five drops of MeOH were also added to the mixture. After stirring overnight, the solvent was carefully evaporated and the remaining white product was washed repeatedly with THF. After drying under vacuum, 174 mg (76.4%) of **45** were obtained as a white foam. R_f(C) = 0.35. M.p. 110-114 °C.

IR (KBr): 699*s*, 740*s*, 1028*s*, 1051*s*, 1093*s*, 1173*s*, 1206*s*, 1250*s*, 1365*s*, 1415*s*, 1454*s*, 1534*s*, 1654*vs*, 1718*vs*, 2946*m*, 2987*m*, 3033*m*, 3293*s*.
^{1}H-NMR (300 MHz, CD_3OD): 1.42, 1.46, 1.51 (3 *br. s*, 2 βCH_3^{Aib}, 2 βCH_3^{Aib}), 1.74-2.02 (*m*, βCH_{2a}^{Gln}, βCH_{2a}^{Hyp}, γCH_2^{Pro}, βCH_{2a}^{Pro}), 2.02-2.18 (*m*, βCH_{2b}^{Gln}, βCH_{2b}^{Pro}), 2.36 (*t*, *J* = 7.4, γCH_2^{Gln}), 2.34-2.48 (*m*, βCH_{2b}^{Hyp}), 3.51 (*d*, *J* = 11.9, δCH_{2a}^{Hyp}), 3.58-3.77 (*m*, δCH_2^{Pro}), 3.68 (*s*, OMe), 3.89 (*d*, *J* = 11.9, δCH_{2b}^{Hyp}), 4.08-4.19 (*m*, αCH^{Gln}, γCH^{Hyp}), 4.38-4.45 (*m*, αCH^{Pro}), 4.46, 4.52 (*AB*, *J* = 11.9, CH_2^{OBn}), 4.57 (*t*, *J* = 8.4, αCH^{Hyp}), 5.04, 5.12 (*AB*, *J* = 12.5, CH_2^{Z}), 7.23-7.38 (*m*, 10 arom. CH).
^{13}C-NMR (75.5 MHz, CD_3OD): 24.6, 24.7 (2 *q,* 2 βCH_3^{Aib}), 25.4, 26.1 (2 *q,* 2 βCH_3^{Aib}), 26.8 (*t,* γCH_2^{Pro}), 28.6 (*t,* βCH_2^{Pro}), 28.9 (*t,* βCH_2^{Gln}), 32.4 (*t,* γCH_2^{Gln}), 34.9 (*t,* βCH_2^{Hyp}), 49.2 (*t,* δCH_2^{Pro}), 52.4 (*q,* OMe), 54.9 (*d,* αCH^{Gln}), 56.2 (*t,* δCH_2^{Hyp}), 57.6, 57.7 (2 *s,* 2 αC^{Aib}), 61.8 (*d,* αCH^{Hyp}), 62.1 (*d,* αCH^{Pro}), 67.7 (*t,* CH_2^{Z}), 71.7 (*t,* CH_2^{OBn}), 78.4 (*d,* γCH^{Hyp}), 128.6, 128.7, 128.8, 129.1, 129.4, 129.5 (6 *d,* 10 arom. CH), 138.1, 139.4 (2 *s,* 2 arom. C), 158.4 (*s,* OCONH^{Z}), 173.7, 174.2, 174.6, 174.7, 177.5 (5 *s,* 6 CO).
ESI-MS (MeOH+NaI): 787.5 (40%, [*M*+Na]$^+$), 765.4 (10%, [*M*+H]$^+$), 636.3 (100%).

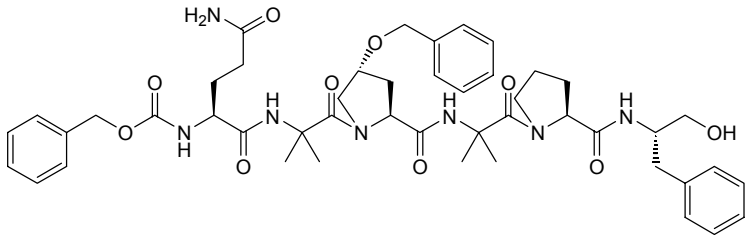
Synthesis of Z-Gln-Aib-Hyp(OBn)-Aib-Pro-OH (46)



According to protocol C, the reaction was carried out with 373.5 mg (0.488 mmol) of **45** and 61.5 mg (1.465 mmol) of $LiOH \cdot H_2O$ dissolved in 23 ml of a mixture of THF, MeOH, and H_2O (3:1:1). The mixture was stirred overnight, and the product was purified by flash chromatography (solvents systems (B) to (C)) and dried in high vacuum to give 276.3 mg (75.4%) of **46** as a white foam. R_f(C) = 0.10. M.p. 135-140 °C.

IR (KBr): 698*m*, 721*m*, 741*m*, 800*m*, 1028*m*, 1088*s*, 1136*s*, 1204*vs*, 1342*s*, 1366*s*, 1427*vs*, 1536*s*, 1667*vs*, 2943*m*, 2988*m*, 3293*s*.
^{1}H-NMR (300 MHz, CD_3OD): 1.42 (*br. s*, βCH_3^{Aib}), 1.47 (2 *br. s*, βCH_3^{Aib}, βCH_3^{Aib}), 1.51 (*br. s*, βCH_3^{Aib}), 1.80-2.02 (*m*, βCH_{2a}^{Gln}, βCH_{2a}^{Hyp}, γCH_2^{Pro}, βCH_{2a}^{Pro}), 2.02-2.17 (*m*, βCH_{2b}^{Gln}, βCH_{2b}^{Pro}), 2.35 (*t*, *J* = 7.2, γCH_2^{Gln}), 2.40-2.48 (*m*, βCH_{2b}^{Hyp}), 3.51 (*d*, *J* = 11.9, δCH_{2a}^{Hyp}), 3.66-3.75 (*m*, δCH_2^{Pro}), 3.88 (*d*, *J* = 11.9, δCH_{2b}^{Hyp}), 4.09-4.18 (*m*, αCH^{Gln}, γCH^{Hyp}), 4.38-4.45 (*m*, αCH^{Pro}), 4.45-4.58 (*m*, CH_2^{OBn}, αCH^{Hyp}), 5.04, 5.11 (*AB*, *J* = 12.5, CH_2^Z), 7.20-7.37 (*m*, 10 arom. CH).
^{13}C-NMR (75.5 MHz, CD_3OD) : 24.6, 24.8 (2 *q,* 2 βCH_3^{Aib}), 25.4, 26.1 (2 *q,* 2 βCH_3^{Aib}), 26.8 (*t,* γCH_2^{Pro}), 28.7 (*t,* βCH_2^{Pro}), 29.1 (*t,* βCH_2^{Gln}), 32.4 (*t,* γCH_2^{Gln}), 34.9 (*t,* βCH_2^{Hyp}), 49.2 (*t,* δCH_2^{Pro}), 54.9 (*d,* αCH^{Gln}), 56.2 (*t,* δCH_2^{Hyp}), 57.7, 57.8 (2 *s,* 2 αC^{Aib}), 61.9 (*d,* αCH^{Hyp}), 62.1 (*d,* αCH^{Pro}), 67.7 (*t,* CH_2^Z), 71.7 (*t,* CH_2^{OBn}), 78.4 (*d,* γCH^{Hyp}), 128.0, 128.2, 128.6, 128.7, 128.9, 129.1, 129.3, 129.4, 129.5 (9 *d,* 10 arom. CH), 138.1, 139.4 (2 *s,* 2 arom. C), 158.9 (*s,* $OCONH^Z$), 173.9, 174.1, 174.6, 176.0, 177.5 (5 *s,* 6 CO).
ESI-MS (MeOH+NaI): 789.5 (60%, $[M+K]^+$), 773.4 (100%, $[M+Na]^+$).

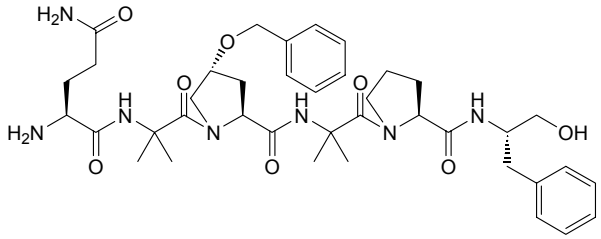
Synthesis of Z-Gln-Aib-Hyp(OBn)-Aib-Pro-Pheol (42)



According to protocol A, a mixture of 152.7 mg (0.203 mmol) of **46**, 30.8 mg (0.203 mmol) of Pheol, 65.3 mg (0.203 mmol) of TBTU, 31.3 mg (0.203 mmol) of HOBt, and 86 µl (0.610 mmol) of TEA in 15 ml of MeCN was stirred 24 h. The product was purified by flash chromatography (solvents systems (B) to (C)) and dried in high vacuum to give 119 mg (66.2%) of **42** as a white foam. R_f(C) = 0.20. M.p. 92-97 °C.

IR (KBr): 700*s*, 743*m*, 1050*s*, 1081*s*, 1149*m*, 1176*m*, 1205*m*, 1248*s*, 1365*s*, 1409*vs*, 1454*s*, 1469*s*, 1543*vs*, 1646*vs*, 1720*s*, 2875*m*, 2940*m*, 2985*m*, 3031*m*, 3062*m*, 3289*vs*.
^{1}H-NMR (300 MHz, CD_3OD): 1.24-1.36 (*m*, βCH_{2a}^{Pro}), 1.43, 1.45 (2 *br. s*, 2 βCH_3^{Aib}), 1.50 (*s*, 2 βCH_3^{Aib}), 1.62-1.74 (*m*, γCH_2^{Pro}), 1.76-1.96 (*m*, βCH_{2a}^{Gln}, βCH_{2a}^{Hyp}), 1.96-2.22 (*m*, βCH_{2b}^{Gln}, βCH_{2b}^{Pro}), 2.33-2.42 (*m*, γCH_2^{Gln}), 2.45-2.57 (*m*, βCH_{2b}^{Hyp}), 2.74 (*dd*, *J* = 13.6, 10.2, $CH_{2a}OH^{Pheol}$), 2.99 (*dd*, *J* = 13.6, 4.7, $CH_{2b}OH^{Pheol}$), 3.44 (*d*, *J* = 12.3, δCH_{2a}^{Hyp}), 3.57-3.63 (*m*, βCH_2^{Pheol}), 3.63-3.79 (*m*, δCH_2^{Pro}), 3.96 (*d*, *J* = 12.1, δCH_{2b}^{Hyp}), 4.07-4.20 (*m*, αCH^{Gln}, γCH^{Hyp}, αCH^{Pheol}), 4.28-4.35 (*m*, αCH^{Pro}), 4.51, 4.55 (*AB*, *J* = 11.8, CH_2^{OBn}), 4.68 (*t*, *J* = 8.9, αCH^{Hyp}), 5.06, 5.15 (*AB*, *J* = 12.7, CH_2^{Z}), 7.22-7.39 (*m*, 15 arom. CH).
^{13}C-NMR (75.5 MHz, CD_3OD): 24.3, 24.7, 26.5 (3 *q,* 4 βCH_3^{Aib}), 26.4 (*t,* γCH_2^{Pro}), 28.7 (*t,* βCH_2^{Pro}), 30.0 (*t,* βCH_2^{Gln}), 32.7 (*t,* γCH_2^{Gln}), 35.5 (*t,* βCH_2^{Hyp}), 37.9 (*t,* CH_2OH^{Pheol}), 50.0 (*t,* δCH_2^{Pro}), 54.6 (*d,* αCH^{Gln}), 55.4 (*t,* δCH_2^{Hyp}), 56.5 (*d,* αCH^{Pheol}), 57.9, 58.0 (2 *s,* 2 αC^{Aib}), 62.2 (*d,* αCH^{Pro}), 64.2 (*d,* αCH^{Hyp}), 65.2 (*t,* βCH_2^{Pheol}), 68.0 (*t,* CH_2^{Z}), 71.9 (*t,* CH_2^{OBn}), 78.8 (*d,* γCH^{Hyp}), 127.2, 128.6, 128.8, 128.9, 129.1, 129.3, 129.4, 129.5, 130.6 (9 *d,* 15 arom. CH), 138.4, 139.6, 140.3 (3 *s,* 3 arom. C), 158.7 (*s,* $OCONH^{Z}$), 174.2, 174.4, 174.6, 175.0, 175.2, 177.7 (6 *s,* 6 CO).
ESI-MS (MeOH+NaI): 906.6 (100%, $[M+Na]^+$).

Synthesis of H-Gln-Aib-Hyp(OBn)-Aib-Pro-Pheol (32)

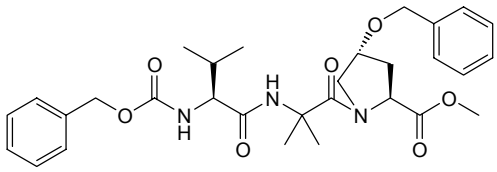


According to protocol D, the reaction was carried out with 528.7 mg (0.598 mmol) of **42** dissolved in 117 ml of MeOH, and 176.2 mg of Pd/C. After drying the product in high vacuum, 448.5 mg (quant.) of **32** were obtained as a yellowish foam. R_f(D) = 0.10. M.p. 77-82 °C.

IR (KBr): 701*m*, 746*m*, 1049*m*, 1081*m*, 1148*m*, 1176*m*, 1204*m*, 1308*m*, 1364*s*, 1409*vs*, 1454*s*, 1470*s*, 1544*vs*, 1645*vs*, 2874*m*, 2939*m*, 2985*m*, 3029*m*, 3062*m*, 3289*vs*.
^{1}H-NMR (300 MHz, CD_3OD): 1.23-1.36 (*m*, βCH_{2a}^{Pro}), 1.44, 1.49 (2 *br. s*, 2 βCH_3^{Aib}), 1.53, 1.54 (2 *br. s*, 2 βCH_3^{Aib}), 1.64-1.76 (*m*, γCH_2^{Pro}), 1.76-1.91 (*m*, βCH_{2a}^{Gln}, βCH_{2a}^{Hyp}), 1.97-2.15 (*m*, βCH_{2b}^{Gln}, βCH_{2b}^{Pro}), 2.38 (*t*, *J* = 8.8, γCH_2^{Gln}), 2.55 (*dd*, *J* = 13.5, 8.5, βCH_{2b}^{Hyp}), 2.75 (*dd*, *J* = 13.9, 10.2, $CH_{2a}OH^{Pheol}$), 2.99 (*dd*, *J* = 13.7, 4.8, $CH_{2b}OH^{Pheol}$), 3.35-3.45 (*m*, δCH_{2a}^{Hyp}), 3.59-3.64 (*m*, βCH_2^{Pheol}), 3.65-3.76 (*m*, δCH_2^{Pro}), 3.76-3.85 (*m*, δCH_{2b}^{Hyp}), 4.09 (*d*, *J* = 11.9, αCH^{Pheol}), 4.11-4.18 (*m*, αCH^{Gln}), 4.18-4.23 (*m*, γCH^{Hyp}), 4.31 (*t*, *J* = 7.6, αCH^{Pro}), 4.53, 4.59 (*AB*, *J* = 12.8, CH_2^{OBn}), 4.69 (*t*, *J* = 8.8, αCH^{Hyp}), 7.22-7.34 (*m*, 10 arom. CH).
^{13}C-NMR (75.5 MHz, CD_3OD): 24.3, 24.8 (2 *q,* 2 βCH_3^{Aib}), 26.4 (*q,* βCH_3^{Aib}), 26.5 (*q,* βCH_3^{Aib}), 26.6 (*t,* γCH_2^{Pro}), 30.8 (*t,* βCH_2^{Pro}), 32.0 (*t,* βCH_2^{Gln}), 32.8 (*t,* γCH_2^{Gln}), 35.6 (*t,* βCH_2^{Hyp}), 37.9 (*t,* CH_2OH^{Pheol}), 50.0 (*t,* δCH_2^{Pro}), 54.6 (*d,* αCH^{Gln}), 55.5 (*d,* αCH^{Pheol}),

55.5 (*t,* δCH_2^{Hyp}), 57.7, 57.9 (2 *s,* 2 αC^{Aib}), 62.3 (*d,* αCH^{Pro}), 64.2 (*d,* αCH^{Hyp}), 65.2 (*t,* βCH_2^{Pheol}), 71.9 (*t,* CH_2^{OBn}), 78.9 (*d,* γCH^{Hyp}), 127.2, 128.6, 128.8, 129.3, 129.5, 130.6 (6 *d,* 10 arom. CH), 139.6, 140.2 (2 *s,* 2 arom. C), 174.4, 174.7, 175.1, 176.9, 178.2 (5 *s,* 6 CO).
ESI-MS (MeOH+NaI): 772.5 (40%, $[M+Na]^+$), 750.4 (50%, $[M+H]^+$), 502.1 (100%).

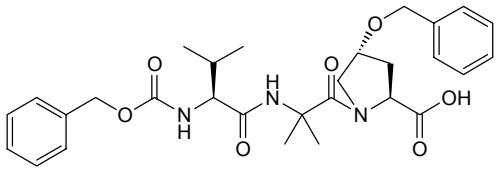
Synthesis of Z-Val-Aib-Hyp(OBn)-OMe (47)



According to protocol B, the reaction was carried out with 189.1 mg (0.752 mmol) of Z-Val-OH and 227.2 mg (0.752 mmol) of **3** dissolved in 10 ml of dry THF. The product was purified by flash chromatography (solvents systems (A) to (C)) and dried in high vacuum to give 321.1 mg (77%) of **47** as a white foam. R_f(C) = 0.80. M.p. 130-134 °C.

IR (KBr): 697*s*, 738*s*, 755*m*, 788*m*, 996*m*, 1011*m*, 1028*s*, 1130*vs*, 1183*vs*, 1212*vs*, 1266*s*, 1280*s*, 1303*m*, 1333*m*, 1363*vs*, 1382*m*, 1418*vs*, 1455*s*, 1470*s*, 1498*m*, 1531*vs*, 1616*vs*, 1677*vs*, 1711*vs*, 1745*vs*, 2893*m*, 2962*s*, 3032*m*, 3064*m*, 3303*m*, 3367*s*.
^{1}H-NMR (300 MHz, CD_3OD): 0.92, 0.96 (2 *d*, *J* = 6.5, 2 γCH_3^{Val}), 1.45, 1.46 (2 *br*. *s*, 2 βCH_3^{Aib}), 1.93-2.07 (*m*, βCH^{Val}, βCH_{2a}^{Hyp}), 2.07-2.20 (*m*, βCH_{2b}^{Hyp}), 3.58, 3.89 (*ABX*, *J* = 10.8, 5.2, δCH_2^{Hyp}), 3.68 (*s*, OMe), 4.00 (*d*, *J* = 7.4, αCH^{Val}), 4.18-4.27 (*m*, γCH^{Hyp}), 4.43-4.54 (*m*, CH_2^{OBn}, αCH^{Hyp}), 4.88, 5.01 (*AB*, *J* = 12.5, CH_2^Z), 7.20-7.37 (*m*, 10 arom. CH).
^{13}C-NMR (75.5 MHz, CD_3OD): 18.6, 19.8 (2 *q,* 2 γCH_3^{Val}), 24.8, 25.4 (2 *q,* 2 βCH_3^{Aib}), 33.2 (*d,* βCH^{Val}), 34.1 (*t,* βCH_2^{Hyp}), 52.6 (*q,* OMe), 53.6 (*t,* δCH_2^{Hyp}), 57.5 (*s,* αC^{Aib}), 60.9 (*d,* αCH^{Hyp}), 61.5 (*d,* αCH^{Val}), 67.6 (*t,* CH_2^Z), 72.2 (*t,* CH_2^{OBn}), 78.1 (*d,* γCH^{Hyp}), 128.6, 128.7, 129.3 (3 *d,* 10 arom. CH), 138.0, 139.4 (2 *s,* 2 arom. C), 158.3 (*s,* $OCONH^Z$), 173.0, 174.2, 174.4 (3 *s,* 3 CO).
ESI-MS (MeOH+NaI): 576.2 (100%, $[M+Na]^+$).

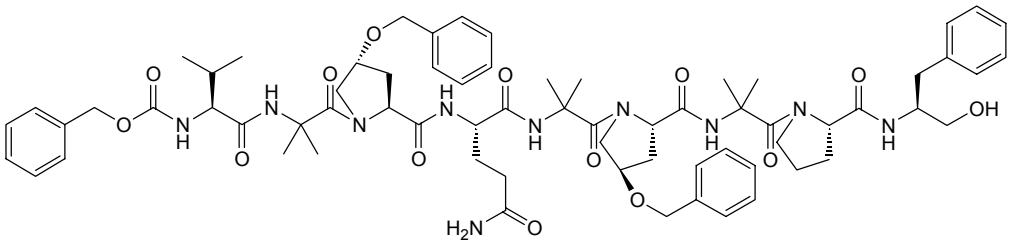
Synthesis of Z-Val-Aib-Hyp(OBn)-OH (43)



According to protocol C, the reaction was carried out with 309.12 mg (0.558 mmol) of **47** and 70.3 mg (1.675 mmol) of $LiOH \cdot H_2O$ dissolved in 19 ml of a mixture of THF, MeOH, and H_2O (3:1:1). The product was purified by preparative TLC (solvents system (D)) and dried in high vacuum to give 193.1 mg (64.1%) of **43** as a white powder. R_f(D) = 0.20. M.p. 125-130 °C.

IR (KBr): 697*s*, 737*s*, 1028*s*, 1095*s*, 1181*s*, 1238*s*, 1270*s*, 1306*s*, 1367*s*, 1430*vs*, 1454*vs*, 1524*vs*, 1616*vs*, 1713*vs*, 2874*m*, 2935*s*, 2965*s*, 3033*m*, 3256*s*.
^{1}H-NMR (300 MHz, $CDCl_3$): 0.88, 0.95 (2 *d*, *J* = 6.7, 2 γCH_3^{Val}), 1.52 (*br. s*, 2 βCH_3^{Aib}), 2.07-2.25 (*m*, βCH^{Val}, βCH_{2a}^{Hyp}), 2.25-2.40 (*m*, βCH_{2b}^{Hyp}), 3.54-3.63 (*m*, δCH_{2b}^{Hyp}), 3.64-3.69 (*m*, δCH_{2a}^{Hyp}), 3.96 (*dd*, *J* = 8.1, 6.3, γCH^{Hyp}), 4.15 (*br. s*, αCH^{Val}), 4.42, 4.52 (*AB*, *J* = 11.8, CH_2^{OBn}), 4.72 (*t*, *J* = 7.8, αCH^{Hyp}), 5.07 (*br. s*, CH_2^{Z}), 7.24-7.38 (*m*, 10 arom. CH).
^{13}C-NMR (75.5 MHz, $CDCl_3$): 17.9 (*q,* γCH_3^{Val}), 19.7 (2 *q,* γCH_3^{Val}, βCH_3^{Aib}), 25.0 (*q,* βCH_3^{Aib}), 30.6 (*d,* βCH^{Val}), 33.0 (*t,* βCH_2^{Hyp}), 53.7 (*t,* δCH_2^{Hyp}), 57.3 (*s,* αC^{Aib}), 60.5 (*d,* αCH^{Hyp}, αCH^{Val}), 67.5 (*t,* CH_2^{Z}), 71.4 (*t,* CH_2^{OBn}), 78.4 (*d,* γCH^{Hyp}), 127.5, 127.9, 128.0, 128.3, 128.5 (5 *d,* 10 arom. CH), 138.1, 139.5 (2 *s,* 2 arom. C), 158.4 (*s,* $OCONH^{Z}$), 173.1, 173.8, 178.8 (3 *s,* 3 CO).
ESI-MS (MeOH+NaI): 562.2 (100%, $[M+Na]^+$).

Synthesis of Z-Val-Aib-Hyp(OBn)-Gln-Aib-Hyp(OBn)-Aib-Pro-Pheol (48)

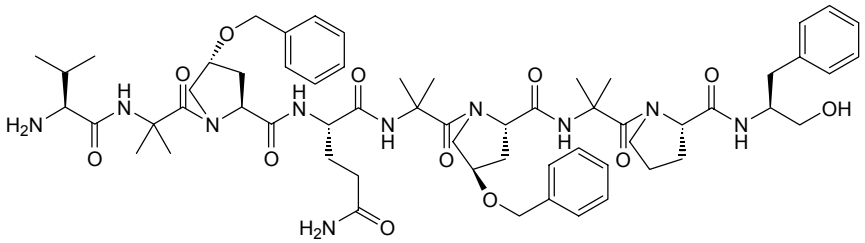


According to protocol A, a mixture of 150 mg (0.2mmol) of **32**, 107.9 mg (0.2 mmol) of **43**, 64.2 mg (0.2 mmol) of TBTU, 30.7 mg (0.2 mmol) of HOBt, and 83.6 µl (0.6 mmol) of TEA in 12 ml of MeCN was stirred for 24 h. The product was purified by preparative TLC (solvents system (C)) and dried in high vacuum to give 127.1 mg (50%) of **48** as a white foam. R_f(C) = 0.40. M.p. 117-124 °C.

IR (KBr): 614*m*, 699*s*, 740*m*, 1028*m*, 1094*s*, 1176*s*, 1205*m*, 1265*s*, 1364*s*, 1411*vs*, 1454*s*, 1470*s*, 1543*vs*, 1647*vs*, 2875*m*, 2936*m*, 3031*m*, 3062*m*, 3291*s*.
^{1}H-NMR (300 MHz, CD_3OD): 0.97, 1.00 (2 *d*, *J* = 7.8, 2 γCH_3^{Val}), 1.21-1.35 (*m*, βCH_{2a}^{Pro}), 1.45, 1.48, 1.49, 1.52, 1.54, 1.57 (6 *br. s*, 6 βCH_3^{Aib}), 1.64-1.74 (*m*, γCH_2^{Pro}), 1.82-1.94 (*m*, 2 βCH_{2a}^{Hyp}), 1.96-2.10 (*m*, βCH_{2b}^{Pro}), 2.10-2.28 (*m*, βCH_{2ab}^{Gln}, βCH^{Val}), 2.29-2.38 (*m*, γCH_2^{Gln}), 2.46-2.61 (*m*, 2 βCH_{2b}^{Hyp}), 2.75, 2.99 (*ABX*, *J* = 13.4, 9.9, CH_2OH^{Pheol}), 3.49 (*ABX*, *J* = 12.6, 3.0, δCH_{2a}^{Hyp}), 3.53-3.60 (*m*, δCH_{2b}^{Hyp}), 3.59-3.64 (*m*, βCH_2^{Pheol}), 3.64-3.85 (*m*, δCH_{2ab}^{Pro}), 3.85-3.93 (*m*, δCH_{2b}^{Hyp}), 4.04 (*d*, *J* = 6.2, αCH^{Val}), 4.09-4.21 (*m*, 2 γCH^{Hyp}, αCH^{Pheol}, δCH_{2a}^{Hyp}), 4.28-4.36 (*m*, αCH^{Gln}, αCH^{Hyp}), 4.46-4.64 (*m*, 2 CH_2^{OBn}, αCH^{Pro}), 4.70 (*t*, *J* = 8.8, αCH^{Hyp}), 5.08, 5.20 (*AB*, *J* = 12.6, CH_2^{Z}), 7.25-7.40 (*m*, 20 arom. CH).
^{13}C-NMR (75.5 MHz, CD_3OD): 17.2, 19.8 (2 *q,* 2 γCH_3^{Val}), 24.1, 24.9, 26.1, 26.3, 26.4, 26.9 (5 *q,* 6 βCH_3^{Aib}; *t,* γCH_2^{Pro}), 28.0 (*t,* βCH_2^{Gln}), 29.8 (*t,* βCH_2^{Pro}), 31.4 (*t,* γCH_2^{Gln}), 33.0 (*d,* βCH^{Val}), 34.8 (*t,* βCH_2^{Hyp}), 35.9 (*t,* βCH_2^{Hyp}), 37.7 (*t,* CH_2OH^{Pheol}), 49.7 (*t,* δCH_2^{Pro}), 54.5 (*d,* αCH^{Gln}), 54.8 (*d,* αCH^{Pheol}), 55.1 (*t,* δCH_2^{Hyp}), 57.7, 57.9 (2 *s,* 3 αC^{Aib}), 62.2 (*d,* αCH^{Hyp}), 62.9 (*d,* αCH^{Hyp}, αCH^{Val}), 63.9 (*d,* αCH^{Pro}), 65.0 (*t,*

βCH$_2^{Pheol}$), 67.8 (*t,* CH$_2^{Z}$), 71.6, 71.7 (2 *t,* 2 CH$_2^{OBn}$), 78.5 (*d,* 2 γCH^{Hyp}), 128.6, 128.7, 128.8, 129.3, 129.4, 129.5, 129.6, 130.6 (8 *d,* 15 arom. CH), 139.4, 140.0 (2 *s,* 4 arom. C), 158.2 (*s,* OCONH^{Z}), 173.7, 174.2, 174.5, 174.8, 174.9, 175.5 (6 *s,* 9 CO).
ESI-MS (MeOH+NaI): 1293.8 (80%, [*M*+Na]$^+$), 658.4 (100%, [*M*+2Na]$^{2+}$).

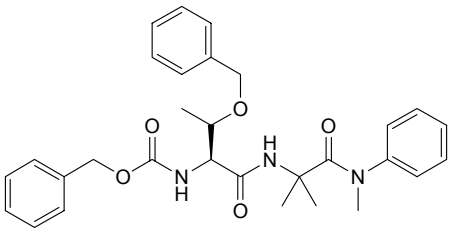
Synthesis of H-Val-Aib-Hyp(OBn)-Gln-Aib-Hyp(OBn)-Aib-Pro-Pheol (49)



According to protocol D, the reaction was carried out with 128.3 mg (0.101 mmol) of **48** in 28.5 ml of MeOH, and 42.7 mg of Pd/C. After drying the product in high vacuum, 101.1 mg (88.1%) of **49** were obtained as a yellowish foam. R_f(C) = 0.15. M.p. 107-113 °C.

IR (KBr): 614*m*, 699*m*, 740*m*, 1028*m*, 1079*s*, 1149*m*, 1176*s*, 1204*m*, 1311*m*, 1364*s*, 1410*vs*, 1454*s*, 1470*s*, 1544*vs*, 1645*vs*, 2873*m*, 2932*s*, 3030*m*, 3062*m*, 3294*vs*.
^{1}H-NMR (600 MHz, CD$_3$OD): 0.91, 1.01 (2 *d*, *J* = 6.9, 2 γCH$_3^{Val}$), 1.21-1.34 (*m*, βCH$_{2a}^{Pro}$), 1.454, 1.504, 1.516, 1.531, 1.552, 1.578 (6 *br. s*, 6 βCH$_3^{Aib}$), 1.64-1.75 (*m*, γCH$_2^{Pro}$), 1.82-1.96 (*m*, 2 βCH$_{2a}^{Hyp}$), 1.98-2.08 (*m*, βCH$_{2b}^{Pro}$), 2.10-2.26 (*m*, βCH$_{2ab}^{Gln}$, βCH^{Val}), 2.26-2.44 (*m*, γCH$_2^{Gln}$), 2.45-2.63 (*m*, 2 βCH$_{2b}^{Hyp}$), 2.75 (*ABX*, *J* = 13.5, 10.1, C*H$_{2a}$*OH^{Pheol}), 2.99 (*ABX*, *J* = 13.7, 5.0, C*H$_{2b}$*OH^{Pheol}), 3.34 (*d*, *J* = 4.6, αCH^{Val}), 3.44-3.52 (*m*, 2 δCH$_{2b}^{Hyp}$), 3.52-3.58 (*m*, 2 δCH$_{2a}^{Hyp}$), 3.59-3.64 (*m*, βCH$_2^{Pheol}$), 3.64-3.86 (*m*, δCH$_{2ab}^{Pro}$), 4.05-4.23 (*m*, 2 δCH$_{2a}^{Hyp}$, γCH^{Hyp}, αCH^{Pheol}), 4.24-4.36 (*m*, γCH^{Hyp}, αCH^{Gln}, αCH^{Pro}), 4.52-4.66 (*m*, 2 CH$_2^{OBn}$, αCH^{Hyp}), 4.70 (*t*, αCH^{Hyp}), 7.23-7.37 (*m*, 20 arom. CH).
^{13}C-NMR (150.9 MHz, CD$_3$OD): 17.2, 19.8 (2 *q,* 2 γCH$_3^{Val}$), 24.0, 24.9, 26.1, 26.3, 27.2 (5 *q,* 6 βCH$_3^{Aib}$; *t,* γCH$_2^{Pro}$), 27.9 (*t,* βCH$_2^{Gln}$), 29.8 (*t,* βCH$_2^{Pro}$), 32.9 (*t,* γCH$_2^{Gln}$), 34.0 (*d,* βCH^{Val}), 35.0 (*t,* βCH$_2^{Hyp}$), 35.5 (*t,* βCH$_2^{Hyp}$), 37.7 (*t,* CH$_2$OH^{Pheol}), 49.7 (*t,* δCH$_2^{Pro}$), 54.4 (*d,* αCH^{Gln}), 54.8 (*d,* αCH^{Pheol}), 55.1 (*t,* 2 δCH$_2^{Hyp}$), 57.4, 57.7 (2 *s,* 3 αC^{Aib}), 62.2 (*d,* αCH^{Hyp}), 62.6 (*d,* αCH^{Hyp}), 64.1 (*d,* αCH^{Pro}), 65.0 (*t,* βCH$_2^{Pheol}$), 68.8 (*d,* αCH^{Val}), 71.6 (*t,* 2 CH$_2^{OBn}$), 78.5, 78.7 (2 *d,* 2 γCH^{Hyp}), 128.6, 128.7, 128.8, 129.3, 129.4, 129.5, 130.6 (7 *d,* 15 arom. CH), 139.4, 140.0 (2 *s,* 3 arom. C), 174.1, 174.5, 174.8, 177.2 (4 *s,* 9 CO).
ESI-MS (MeOH): 1137.5 (100%, [M+H]$^+$).

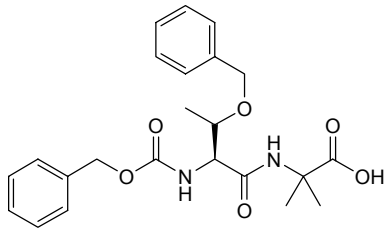
Synthesis of Z-Thr(OBn)-Aib-N(Me)(Ph) (38)



According to protocol B, the reaction was carried out with 138 mg (0.402 mmol) of Z-Thr(OBn)-OH and 70 mg (0.402 mmol) of **1** dissolved in 16 ml of CH_2Cl_2. After 48 h, the mixture was purified by preparative TLC (solvents system CH_2Cl_2/AcOEt (1.5:1)) and dried in high vacuum to give 208.4 mg (quant.) of **38** as a white foam. R_f(A) = 0.70. M.p. 134-135 °C.

IR (KBr): 698*s*, 709*s*, 739*s*, 761*s*, 773*s*, 983*s*, 1027*m*, 1068*s*, 1095*s*, 1170*m*, 1214*vs*, 1286*s*, 1336*m*, 1360*m*, 1375*m*, 1397*s*, 1454*s*, 1467*s*, 1495*vs*, 1593*s*, 1630*vs*, 1676*vs*, 1721*vs*, 2896*m*, 2929*m*, 2969*m*, 2983*m*, 3027*m*, 3309*s*, 3421*s*.
^{1}H-NMR (300 MHz, $CDCl_3$): 1.09 (*d*, *J* = 6.5, γCH_3^{Thr}), 1.38, 1.54 (2 *br*. *s*, 2 βCH_3^{Aib}), 3.24 (*br*. *s*, $CH_3^{N(Me)}$), 3.82-3.89 (*m*, αCH^{Thr}), 3.94-4.03 (*m*, βCH^{Thr}), 4.37, 4.51 (*AB*, *J* = 11.4, CH_2^{OBn}), 5.10, 5.17 (*AB*, *J* = 12.2, CH_2^{Z}), 7.20-7.43 (*m*, 10 arom. CH).
^{13}C-NMR (75.5 MHz, $CDCl_3$): 14.8 (*q,* γCH_3^{Thr}), 26.1, 27.1 (2 *q,* 2 βCH_3^{Aib}), 41.2 (*q,* $CH_3^{N(Me)}$), 56.9 (*d,* αCH^{Thr}), 57.9 (*s,* αC^{Aib}), 66.8 (*t,* CH_2^{Z}), 71.4 (*t,* CH_2^{OBn}), 74.4 (*d,* βCH^{Thr}), 127.8, 127.9, 128.1, 128.3, 128.5, 128.7, 129.5 (7 *d,* 15 arom. CH), 136.3, 137.7 (2 *s,* 2 arom. C), 144.6 (*s,* arom. $C^{N(Ph)}$), 156.0 (*s,* $OCONH^{Z}$), 169.6, 172.5 (2 *s,* 2 CO).
ESI-MS (MeOH+NaI): 540.6 (10%, $[M+Na]^+$), 518.2 (27%, $[M+H]^+$), 411.1 (100%), 303.0 (60%).

Synthesis of Z-Thr(OBn)-Aib-OH (39)

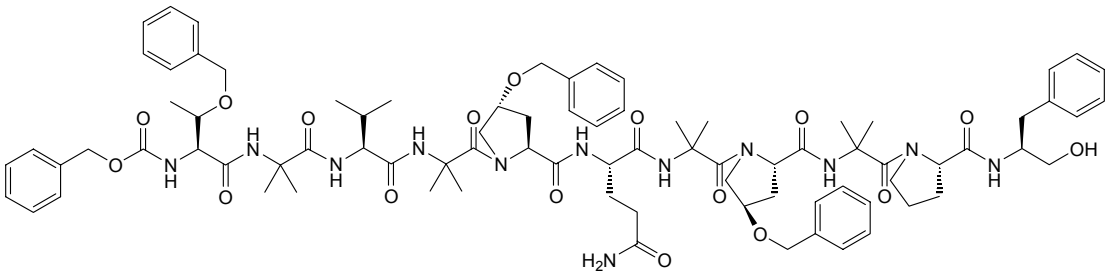


According to protocol E, the reaction was carried out with 674.3mg (1.304 mmol) of **38** in a mixture of 12 ml of 6N HCl and 12 ml of THF. The temperature was allowed to rise from 0 °C to room temperature overnight. After two times extraction with CH_2Cl_2, the organic layer was dried with $MgSO_4$, filtered, the solvent was evaporated and the product was dried in high vacuum to give 560.2 mg (quant.) of **39** as a white foam. R_f(A) = 0.33. M.p. 90-92 °C.

IR (KBr): 697*vs*, 737*s*, 1028*s*, 1069*vs*, 1174*s*, 1215*vs*, 1360*vs*, 1405*vs*, 1456*vs*, 1498*vs*, 1605*vs*, 1668*vs*, 1718*vs*, 2873*m*, 2933*s*, 2978*s*, 3033*m*, 3065*m*, 3349*s*.

^{1}H-NMR (300 MHz, $CDCl_3$): 1.17 (*d*, *J* = 6.5, γCH_3^{Thr}), 1.48, 1.49 (2 *br*. *s*, 2 βCH_3^{Aib}), 4.03-4.13 (*m*, αCH^{Thr}), 4.34-4.41 (*m*, βCH^{Thr}), 4.48, 4.56 (*AB*, *J* = 11.6, CH_2^{OBn}), 5.09 (*br*. *s*, CH_2^{Z}), 7.22-7.34 (*m*, 10 arom. CH).
^{13}C-NMR (75.5 MHz, $CDCl_3$): 14.9 (*q,* γCH_3^{Thr}), 28.9, 29.7 (2 *q,* 2 βCH_3^{Aib}), 56.3 (*d,* αCH^{Thr}), 57.4 (*s,* αC^{Aib}), 67.0 (*t,* CH_2^{Z}), 71.4 (*t,* CH_2^{OBn}), 74.6 (*d,* βCH^{Thr}), 128.1, 128.2, 128.3, 128.5, 128.8, 128.9 (6 *d,* 10 arom. CH), 136.0, 137.7 (2 *s,* 2 arom. C), 156.2 (*s,* $OCONH^{Z}$), 169.1, 177.8 (2 *s,* 2 CO).
ESI-MS (MeOH+NaI): 923.4 (13%, $[2M\text{-}2H\text{+}3Na]^+$), 901.3 (17%, $[2M\text{-}H\text{+}2Na]^+$), 879.4 (5%, $[2M\text{+}Na]^+$), 451.1 (100%, $[M\text{+}Na]^+$).

Synthesis of Z-Thr(OBn)-Aib-Val-Aib-Hyp(OBn)-Gln-Aib-Hyp(OBn)-Aib-Pro-Pheol (4)

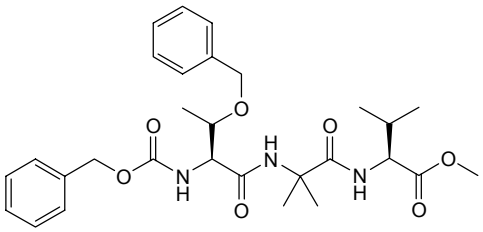


According to protocol A, the reaction was carried out with 94 mg (0.083 mmol) of **49**, 35.4 mg (0.083 mmol) of **39**, 26.5 mg (0.083 mmol) of TBTU, 12.7 mg (0.083 mmol) of HOBt, and 34.9 µl (0.248 mmol) of TEA in 8 ml of MeCN. The product was purified by flash chromatography (solvents systems (B) to (D)) and dried in high vacuum to give 42 mg (32.8%) of **4** as a yellow foam. R_f(D) = 0.70. M.p. 115-120 °C.

IR (KBr): 699*m*, 740*m*, 1028*m*, 1078*s*, 1176*s*, 1207*m*, 1275*m*, 1364*s*, 1383*s*, 1410*s*, 1454*s*, 1469*s*, 1540*vs*, 1645*vs*, 2873*m*, 2934.83*m*, 2984*m*, 3031*m*, 3063*m*, 3293*s*.
^{1}H-NMR (300 MHz, CD_3OD): 0.88, 0.90 (2 *d*, *J* = 2.7, 2 γCH_3^{Val}), 1.23-1.34 (*m*, βCH_{2a}^{Pro}, γCH_3^{Thr}), 1.41, 1.46, 1.50, 1.51, 1.51, 1.54, 1.56, 1.58 (8 *br. s,* 8 βCH_3^{Aib}), 1.63-1.74 (*m*, γCH_2^{Pro}), 1.81-2.09 (*m*, 2 βCH_{2b}^{Hyp}, βCH_{2b}^{Pro}, βCH_{2b}^{Gln}), 2.10-2.18 (*m*, βCH^{Val}, βCH_{2a}^{Gln}), 2.20-2.38 (*m*, γCH_2^{Gln}), 2.45-2.58 (*m*, 2 βCH_{2b}^{Hyp}), 2.75 (*ABX*, *J* = 13.6, 10.0, $CH_{2a}OH^{Pheol}$), 2.98 (*ABX*, *J* = 13.5, 4.9, $CH_{2b}OH^{Pheol}$), 3.50 (*ABX*, *J* = 12.4, 2.7, δCH_{2b}^{Hyp}), 3.54-3.66 (*m*, δCH_{2b}^{Hyp}, βCH_2^{Pheol}), 3.66-3.75 (*m*, δCH_{2a}^{Pro}), 3.75-3.86 (*m*, δCH_{2b}^{Pro}), 4.01-4.07 (*m*, δCH_{2a}^{Hyp}), 4.10-4.36 (*m*, αCH^{Val}, αCH^{Gln}, αCH^{Pro}, 2 γCH^{Hyp}, αCH^{Pheol}, δCH_{2a}^{Hyp}, αCH^{Thr}, βCH^{Thr}), 4.45-4.75 (*m*, 2 αCH^{Hyp}, 3 CH_2^{OBn}), 5.14, 5.21 (*AB*, *J* = 12.7, CH_2^{Z}), 7.23-7.40 (*m*, 25 arom. CH).
^{13}C-NMR (75.5 MHz, CD_3OD): 16.6 (*q,* γCH_3^{Thr}), 18.5, 19.7 (2 *q,* 2 γCH_3^{Val}), 24.0, 24.1, 24.2, 24.9, 26.1, 26.3, 27.0, 27.6 (7 *q,* 8 βCH_3^{Aib}; *t,* γCH_2^{Pro}), 27.9 (*t,* βCH_2^{Gln}), 29.8 (*t,* βCH_2^{Pro}), 31.1 (*t,* γCH_2^{Gln}), 32.7 (*d,* βCH^{Val}), 35.4 (*t,* βCH_2^{Hyp}), 35.8 (*t,* βCH_2^{Hyp}), 37.7 (*t,* CH_2OH^{Pheol}), 49.7 (*t,* δCH_2^{Pro}), 54.4 (*d,* αCH^{Gln}), 54.8 (*d,* αCH^{Pheol}), 54.8, 55.1 (2 *t,* 2 δCH_2^{Hyp}), 57.7, 57.8, 58.1 (3 *s,* 4 αC^{Aib}), 60.3 (*d,* αCH^{Hyp}), 62.2 (*d,* αCH^{Hyp}), 62.9 (*d,* αCH^{Pro}), 63.3 (*d,* αCH^{Thr}), 63.9 (*d,* αCH^{Val}), 65.0 (*t,* βCH_2^{Pheol}), 68.0 (*t,* CH_2^{Z}), 71.6, 72.1 (3 *t,* 3 CH_2^{OBn}), 75.0 (*d,* βCH^{Thr}), 78.4 (*d,* γCH^{Hyp}), 128.5, 128.7, 128.9, 129.0, 129.1, 129.3, 129.4, 129.6, 130.6 (9 *d,* 25 arom. CH), 137.3, 139.3, 139.4,

140.0 (4 *s,* 5 arom. C), 159.2 (*s,* $OCONH^{Z}$), 173.7, 174.1, 174.5, 174.8, 175.0, 175.7, 177.1 (7 *s,* 11 CO).
ESI-MS (MeOH+NaI): 1569.9 (100%, $[M+Na]^{+}$), 796.7 (60%, $[M+2Na]^{2+}$).

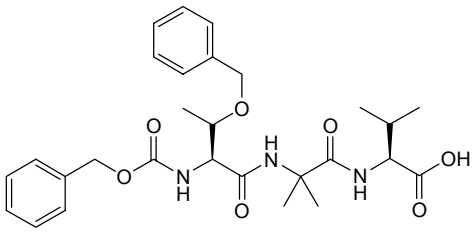
Synthesis of Z-Thr(OBn)-Aib-Val-OMe (40)



According to protocol A, the reaction was carried out with 525.2 mg (1.2257 mmol) of **39**, 205.5 mg (1.2257 mmol) of H-Val-OMe·HCl, 393.6 mg (1.2257 mmol) of TBTU, 188.1 mg (1.2257 mmol) of HOBt, and 510 µl (3.6771 mmol) of TEA in 42 ml of MeCN. The product was purified by flash chromatography (solvents systems (Hexan/CH_2Cl_2 1:1) to (A)) and dried in high vacuum to give 569.5 mg (86%) of **40** as a white foam. R_f(ethyl acetate/CH_2Cl_2 1:1) = 0.48.

^{1}H-NMR (300 MHz, CD_3OD): 0.85, 0.89 (2 *d*, *J* = 6.7, 2 γCH_3^{Val}), 1.22 (*d*, *J* = 6.1, γCH_3^{Thr}), 1.44, 1.47 (2 *br. s,* 2 βCH_3^{Aib}), 2.00-2.12 (*m*, βCH^{Val}), 3.68 (*s*, OMe), 3.90-3.98 (*m*, βCH^{Thr}), 4.19 (*d*, *J* = 5.0, αCH^{Thr}), 4.25 (*d*, *J* = 6.1, αCH^{Val}), 4.50, 4.61 (*AB*, *J* = 11.6, CH_2^{OBn}), 5.11 (*br. s*, CH_2^{Z}), 7.25-7.36 (*m*, 10 arom. CH).
^{13}C-NMR (75.5 MHz, CD_3OD): 16.5 (*q,* γCH_3^{Thr}), 18.9, 19.5 (2 *q,* 2 γCH_3^{Val}), 24.7, 26.2 (2 *q,* 2 βCH_3^{Aib}), 31.8 (*d,* βCH^{Val}), 52.4 (*q,* CH_3^{OMe}), 58.2 (*s,* αC^{Aib}), 59.6 (*d,* αCH^{Val}), 61.1 (*d,* αCH^{Thr}), 67.8 (*t,* CH_2^{Z}), 72.2 (*t,* CH_2^{OBn}), 76.0 (*d,* βCH^{Thr}), 128.7, 128.8, 128.9, 129.0, 129.3, 129.5 (6 *d,* 10 arom. CH), 138.1, 139.6 (2 *s,* 2 arom. C), 158.6 (*s,* $OCONH^{Z}$), 172.0, 173.5, 176.6 (3 *s,* 3 CO).

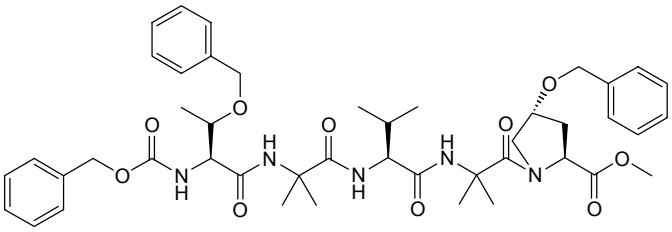
Synthesis of Z-Thr(OBn)-Aib-Val-OH (41)



According to protocol C, the reaction was carried out with 516.1 mg (0.9528 mmol) of **40** and 120.1 mg (2.8585 mmol) of $LiOH·H_2O$ dissolved in 40 ml of a mixture of THF, MeOH, and H_2O (3:1:1). The product was purified by repeated flash column chromatography and dried in high vacuum to give 165 mg (32.8%) of **41** as a white foam. R_f(B) = 0.51.

^{1}H-NMR (300 MHz, CD_3OD): 0.86, 0.92 (2 *d*, *J* = 6.7, 2 γCH_3^{Val}), 1.24 (*d*, *J* = 3.4, γCH_3^{Thr}), 1.45, 1.48 (2 *br. s,* 2 βCH_3^{Aib}), 2.08-2.20 (*m*, βCH^{Val}), 3.96-4.05 (*m*, βCH^{Thr}), 4.23 (*d*, *J* = 3.8, αCH^{Thr}), 4.10 (*dd*, *J* = 14.1, 7.1, αCH^{Val}), 4.49, 4.61 (*AB*, *J* = 11.6, CH_2^{OBn}), 5.14 (*br. s*, CH_2^{Z}), 7.25-7.40 (*m*, 10 arom. CH).
^{13}C-NMR (75.5 MHz, CD_3OD): 16.6 (*q,* γCH_3^{Thr}), 18.6, 19.6 (2 *q,* 2 γCH_3^{Val}), 24.7, 26.2 (2 *q,* 2 βCH_3^{Aib}), 31.8 (*d,* βCH^{Val}), 58.3 (*s,* αC^{Aib}), 59.4 (*d,* αCH^{Val}), 60.0 (*d,* αCH^{Thr}), 67.8 (*t,* CH_2^{Z}), 72.3 (*t,* CH_2^{OBn}), 76.0 (*d,* βCH^{Thr}), 128.7, 128.8, 128.9, 129.0, 129.3, 129.5 (6 *d,* 10 arom. CH), 138.1, 139.6 (2 *s,* 2 arom. C), 158.6 (*s,* $OCONH^{Z}$), 172.1, 174.9, 176.5 (3 *s,* 3 CO).

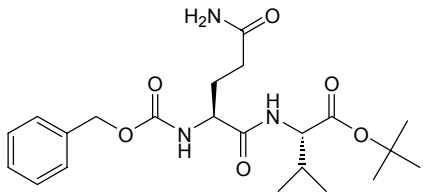
Synthesis of Z-Thr(OBn)-Aib-Val-Aib-Hyp(OBn)-OMe (37)



According to protocol B, the reaction was carried out with 56 mg (0.106 mmol) of **41** and 34.3 mg (1.114 mmol) of **3** dissolved in 4 ml of THF. The product was purified by flash chromatography (solvents systems (A) to (C)) and dried in high vacuum to give 74.6 mg (84.8%) of **37** as a white foam. R_f(B) = 0.80. M.p. 75 °C.

IR (KBr): 3327*s*, 3064*m*, 3033*m*, 2961*m*, 2874*m*, 1671*vs*, 1529*vs*, 1455*s*, 1415*s*, 1382*s*, 1362*s*, 1272*s*, 1214*s*, 1175*s*, 1082*s*, 1028*m*, 739*m*, 698*m*.
^{1}H-NMR (300 MHz, CD_3OD): 0.84, 0.86 (2 *d*, *J* = 4.5, 2 γCH_3^{Val}), 1.25 (*d*, *J* = 6.3, γCH_3^{Thr}), 1.38, 1.45, 1.48 (3 *br. s*, 2 βCH_3^{Aib}, 2 βCH_3^{Aib}), 1.91-2.02 (*m*, βCH^{Val}), 2.13-2.27 (*m*, βCH_2^{Hyp}), 3.69 (*s*, CH_3^{OMe}), 3.71-3.76 (*m*, δCH_{2b}^{Hyp}), 3.86-4.02 (*m*, δCH_{2a}^{Hyp}, αCH^{Thr}), 4.02-4.09 (*m*, αCH^{Val}), 4.19-4.28 (*m*, βCH^{Thr}, γCH^{Hyp}), 4.41-4.63 (*m*, $CH_2^{Thr(OBn)}$, $CH_2^{Hyp(OBn)}$, αCH^{Hyp}), 5.09, 5.16 (*AB*, *J* = 12.7, CH_2^{Z}), 7.1-7.5 (*m*, 15 arom. H).
^{13}C-NMR (75.5 MHz, CD_3OD): 16.7 (*q,* γCH_3^{Thr}), 18.3, 20.0 (2 *q,* 2 γCH_3^{Val}), 24.9, 25.2, 25.8, 26.7 (4 *q,* 4 βCH_3^{Aib}), 31.3 (*d,* βCH^{Val}), 34.6 (*t,* βCH_2^{Hyp}), 52.5 (*q,* CH_3^{OMe}), 54.2 (*t,* δCH_2^{Hyp}), 57.7, 58.2 (2 *s,* 2 αC^{Aib}), 59.8 (*d,* αCH^{Val}), 61.3 (*d,* αCH^{Thr}), 62.6 (*d,* αCH^{Hyp}), 67.9 (*t,* CH_2^{Z}), 72.0, 72.2 (2 *t,* 2 CH_2^{OBn}), 75.4 (*t,* γCH^{Hyp}), 78.7 (*d,* βCH^{Thr}), 128.6, 128.7, 128.9, 129.0, 129.4, 129.5 (6 *d,* 15 arom. CH), 138.1, 139.4 (2 *s,* 3 arom. C), 159.0 (*s,* $OCONH^{Z}$), 172.6, 172.9, 174.4, 174.6, 176.7 (5 *s,* 5 CO).

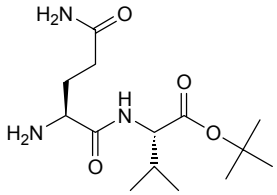
Synthesis of Z-Gln-Val-O*t*Bu (53)



According to protocol A, to Z-Gln-OH (2.5 g, 8.93 mmol) and H-Val-O*t*Bu·HCl (1.871 g, 8.93 mmol) were added HOBt (1.507 g, 8.93 mmol) and TBTU (2.867 g, 8.93 mmol). The mixture was completely dissolved in MeCN (560 ml) and TEA (3.76 ml, 26.78 mmol) was added. After the completion of the reaction, the mixture was purified by flash chromatography to lead to the dipeptide **53** (3.108 g, 80%). White powder looking like plaster. Rather insoluble in MeOH at r.t. M.p. 149 °C. R_f(B) = 0.78. RP-HPLC (II): t_R 21.07, λ 210.

IR (KBr): 3445*m*, 3326*s*, 2964*m*, 1720*s*, 1686*s,* 1652*vs,* 1529*s,* 1448*m,* 1414*m,* 1393*m,* 1371*m,* 1351*m,* 1286*m,* 1258*s,* 1241*s,* 1216*m,* 1161*m,* 1136*m,* 1042*m,* 698*m*.
^{1}H-NMR (300 MHz, CD_3OD): 0.95 (*d*, *J* = 6.6, 2 γCH_3^{Val}), 1.45 (*br. s*, 3 CH_3^{OtBu}), 1.82-1.95 (*m*, βCH_{2a}^{Gln}), 2.00-2.21 (*m*, βCH^{Val}, βCH_{2b}^{Gln}), 2.34 (*t*, *J* = 7.4, γCH_2^{Gln}), 4.19-4.25 (*m*, αCH^{Gln}, αCH^{Val}), 5.08 (*s*, CH_2^Z), 7.30-7.46 (*m*, 5 arom. CH^Z).
^{13}C-NMR (75.5 MHz, CD_3OD): 18.3, 19.4 (2 *q*, 2 γCH_3^{Val}), 28.2 (*q*, 3 CH_3^{OtBu}), 29.2 (*t*, βCH_2^{Gln}), 31.7 (*d*, βCH^{Val}), 38.8 (*t*, γCH_2^{Gln}), 55.6 (*d*, αCH^{Gln}), 59.7 (*d*, αCH^{Val}), 67.7 (*t*, CH_2^Z), 82.9 (*s*, C^{OtBu}), 128.9, 129.1, 129.4 (3 *d*, 5 arom. CH^Z), 138.0 (*s*, arom. C^Z), 158.3 (*s*, $OCONH^Z$), 172.1, 174.3, 177.7 (3 *s*, 3 CO).
ESI-MS (MeOH+NaI): 893.5 (35%, $[2M+Na]^+$), 458.2 (100%, $[M+Na]^+$).
Anal. calc. for $C_{22}H_{33}N_3O_6$ (MG 435.16): C 60.67, H 7.64, N 9.65; found: C 60.27, H 7.64, N 9.59.
$[\alpha]_D^{23} = -12.1$ (*c* = 0.63, MeCN).

Synthesis of H-Gln-Val-O*t*Bu (55)

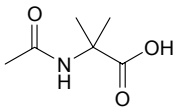


According to protocol D, the dipeptide **53** (760 mg, 1.745 mmol) was dissolved in MeOH (60 ml). Pd/C was added and the reaction was left overnight under H_2 atmosphere. After the end of the reaction and filtration on *Celite*, the solvent was evaporated leading to the N-unprotected dipeptide **55** (525 mg, quant.). Sticky, colorless oil. R_f(B) = 0.05. RP-HPLC (II): t_R 23.61, λ 201.

IR (KBr): 3418*vs,* 2971*s,* 2934*s,* 1730*vs,* 1666*vs,* 1538*s,* 1491*m,* 1455*m,* 1394*s,* 1370*s,* 1314*m,* 1258*m,* 1151*vs,* 598*m*.

^{1}H-NMR (300 MHz, CD_3OD): 0.93 (*d*, *J* = 6.9, 2 γCH_3^{Val}), 1.44 (*br. s*, 3 CH_3^{OtBu}), 1.71-1.86 (*m*, βCH_{2a}^{Gln}), 1.91-2.04 (*m*, βCH^{Val}), 2.06-2.18 (*m*, βCH_{2b}^{Gln}), 2.31 (*t*, *J* = 7.3, γCH_2^{Gln}), 3.42 (*t*, *J* = 6.9, αCH^{Gln}), 4.18 (*d*, *J* = 5.2, αCH^{Val}).
^{13}C-NMR (75.5 MHz, CD_3OD): 18.4, 19.5 (2 *q*, 2 γCH_3^{Val}), 28.3 (*q*, 3 CH_3^{OtBu}), 31.7 (*d*, βCH^{Val}), 32.4, 32.7 (2 *t*, βCH_2^{Gln}, γCH_2^{Gln}), 55.2 (*d*, αCH^{Gln}), 59.5 (*d*, αCH^{Val}), 82.8 (*s*, C^{OtBu}), 172.2, 177.1, 178.0 (3 *s*, 3 CO).
ESI-MS (MeOH+NaI): 625.3 (15%, $[2M+Na]^+$), 324.2 (100%, $[M+Na]^+$).
$[\alpha]_D^{20} = -14.5$ (*c* = 1, MeOH).

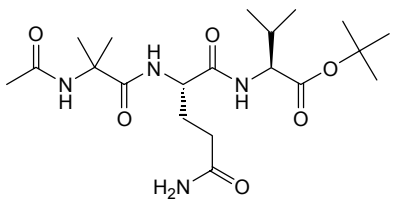
Synthesis of Ac-Aib-OH



A mixture of H-Aib-OH (2-Methylalanin; 430 mg, 4.1748 mmol), acetic acid anhydride (1431 mg, 13.2504 mmol) and pyridine (1047 mg, 13.2504 mmol) was stirred for 17 h at room temperature and then was heated to reflux for 1 h. Ice was added to the solution to reach 0 °C, and the mixture was stirred at that temperature for 4 h. The solution was evaporated, the solid residue was washed with ether, and recrystallized in MeOH to give Ac-Aib-OH (424.9 mg, 70%). White crystal material. R_f(A) = 0.29.

^{1}H-NMR (300 MHz, CD_3OD): 1.91 (*s*, CH_3^{Ac}), 1.46 (*br. s*, 2 βCH_3^{Aib}).
^{13}C-NMR (75.5 MHz, CD_3OD): 23.1 (*q*, CH_3^{Ac}), 26.0 (2 *q*, 2 βCH_3^{Aib}), 57.5 (*s*, αC^{Aib}), 173.2, 178.6 (2 *s*, 2 CO).

Synthesis of Ac-Aib-Gln-Val-OtBu (57)

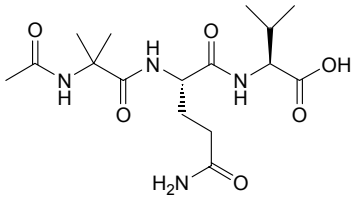


According to protocol A, to the N-unprotected dipeptide **55** (580 mg, 1.92 mmol) and Ac-Aib-OH (279 mg, 1.92 mmol) were added HOBt (295 mg, 1.92 mmol) and TBTU (618 mg, 1.92 mmol). The mixture was completely dissolved in MeCN (111 ml) and TEA (811 µl, 5.77 mmol) was added. After completion of the reaction, the mixture was purified by flash chromatography to lead to the tripeptide **57** (555 mg, 67%). White foam. R_f(B) = 0.22, R_f(C) = 0.74. M.p. 83-86 °C. RP-HPLC (II): t_R 36.46, λ 206.

IR (KBr): 3313*s,* 2975*m,* 2934*m,* 1729*s,* 1663*vs,* 1535*vs,* 1452*m,* 1369*s,* 1294*s,* 1220*m,* 1149*s,* 1084*s*.
^{1}H-NMR (300 MHz, CD_3OD): 0.96 (*d*, *J* = 6.9, 2 γCH_3^{Val}), 1.44, 1.46 (2 *br. s*, 2 βCH_3^{Aib}), 1.47 (*br. s*, 3 CH_3^{OtBu}), 1.94-2.06 (*m*, βCH_{2a}^{Gln}), 1.97 (*s*, CH_3^{Ac}), 2.10-2.21 (*m*,

βCH^{Val}, βCH$_{2b}$^{Gln}), 2.32 (*t*, *J* = 7.6, γCH$_2$^{Gln}), 4.11 (*d*, *J* = 6.4, αCH^{Val}), 4.37 (*dd*, *J* = 9.6, 4.6, αCH^{Gln}).
^{13}C-NMR (75.5 MHz, CD$_3$OD): 18.8, 19.5 (2 *q*, 2 γCH$_3$^{Val}), 23.1, 24.5 (2 *q*, 2 βCH$_3$^{Aib}), 26.7 (*q*, CH$_3$^{Ac}), 28.3 (*q*, 3 CH$_3$^{OtBu}; *t*, βCH$_2$^{Gln}), 31.5 (*d*, βCH^{Val}), 32.5 (*t*, γCH$_2$^{Gln}), 54.4 (*d*, αCH^{Gln}), 57.8 (*s*, αC^{Aib}), 60.3 (*d*, αCH^{Val}), 82.9 (*s*, C^{OtBu}), 172.1, 173.2, 174.1, 177.1, 178.2 (5 *s*, 5 CO).
ESI-MS (MeOH+NaI): 467.1 (3%, [*M*+K]$^+$), 451.2 (60%, [*M*+Na]$^+$), 429.2 (90%, [*M*+H]$^+$), 373.2 (100%).
Anal. calc. for C$_{20}$H$_{36}$N$_4$O$_6$ · 0.5 H$_2$O (MG 437.53): C 54.90, H 8.52, N 12.81; found: C 55.06, H 8.38, N 12.68.
$[\alpha]_D^{20}$ = –32.5 (*c* = 1.75, AcOEt).

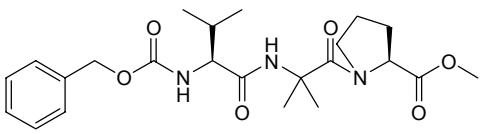
Synthesis of Ac-Aib-Gln-Val-OH (51)



At 0 °C, the tripeptide *tert*-butyl ester **57** (260 mg, 0.61 mmol) was dissolved in TFA (15 ml) and left for 2.5 h under an N$_2$ atmosphere. The reaction was quenched by evaporating TFA. The residue was dissolved in a mixture of CH$_2$Cl$_2$/Et$_2$O 1/1 (v/v) and evaporated (3×) to lead to the C-unprotected tripeptide **51** (225 mg, quant.). White powder. R_f(C) = 0.28. M.p. 70-74 °C. RP-HPLC (II): t_R 16.00, λ 215.

IR (KBr): 3474*vs,* 3364*vs,* 3330*vs,* 2973*m,* 2936*m,* 1653*vs,* 1525*vs,* 1468*m,* 1433*m,* 1392*m,* 1370*m,* 1290*s,* 1197*s,* 1130*s,* 1083*m,* 1036*m,* 619*m*.
^{1}H-NMR (300 MHz, CD$_3$OD): 0.98 (*d*, *J* = 6.9, 2 γCH$_3$^{Val}), 1.44, 1.45 (2 *br*. *s*, 2 βCH$_3$^{Aib}), 1.90-2.03 (*m*, βCH$_{2a}$^{Gln}), 1.97 (*s*, CH$_3$^{Ac}), 2.09-2.26 (*m*, βCH^{Val}, βCH$_{2b}$^{Gln}), 2.32 (*t*, *J* = 6.8, γCH$_2$^{Gln}), 4.26 (*d*, *J* = 5.7, αCH^{Val}), 4.38 (*dd*, *J* = 9.6, 4.3, αCH^{Gln}).
^{13}C-NMR (75.5 MHz, CD$_3$OD): 18.6, 19.6 (2 *q*, 2 γCH$_3$^{Val}), 23.1, 25.3 (2 *q*, 2 βCH$_3$^{Aib}), 25.8 (*q*, CH$_3$^{Ac}), 28.3 (*t*, βCH$_2$^{Gln}), 31.6 (*d*, βCH^{Val}), 32.5 (*t*, γCH$_2$^{Gln}), 54.5 (*d*, αCH^{Gln}), 57.7 (*s*, αC^{Aib}), 59.3 (*d*, αCH^{Val}), 173.3, 174.1, 174.5, 177.2, 178.2 (5 *s*, 5 CO).
ESI-MS (MeOH+NaI): 395.3 (100%, [*M*+Na]$^+$), 767.4 (10%, [2*M*+Na]$^+$), 373.3 (18%, [*M*+H]$^+$).
$[\alpha]_D^{20}$ = –13.2 (*c* = 1, MeOH).

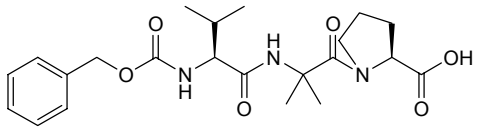
Synthesis of Z-Val-Aib-Pro-OMe (61)



According to protocol B, the 2*H*-azirin-3-amine **2** (230.4 mg, 1.176 mmol) was dissolved in CH_2Cl_2 (20 ml), Z-Val-OH (246.2 mg, 0.98 mmol) was added and the mixture was stirred overnight. The solvent was evaporated and the product purified by flash chromatography to give the C-protected tripeptide **61** (426.8 mg, 97%). White foam. R_f(A) = 0.58. M.p. 57-59 °C. RP-HPLC (II): t_R 41.68, λ 216.

IR (KBr): 3311*vs,* 3065*m,* 3035*m,* 2964*s,* 2877*m,* 1745*vs,* 1662*vs,* 1620*vs,* 1538*vs,* 1470*vs,* 1455*vs,* 1414*vs,* 1364*vs,* 1342*s,* 1289*vs,* 1242*vs,* 1170*vs*, 1117*m,* 1095*m,* 1027*s,* 742*m,* 698*s,* 640*m,* 614*m*.
^{1}H-NMR (300 MHz, CD_3OD): 0.94, 0.97 (2 *d*, *J* = 6.9, 2 γCH_3^{Val}), 1.43, 1.45 (2 *br*. *s*, 2 βCH_3^{Aib}), 1.62-2.03 (*m*, βCH^{Val}, βCH_2^{Pro}, γCH_2^{Pro}), 3.48-3.67 (*m*, δCH_2^{Pro}), 3.67 (*s*, OMe), 3.89-3.95 (*m*, αCH^{Val}), 4.38 (*dd*, *J* = 8.4, 3.8, αCH^{Pro}), 5.04, 5.10 (*AB*, *J* = 12.6, CH_2^Z), 7.04 (*d*, *J* = 8.8, NH^{Val}), 7.28-7.36 (*m*, 5 arom. CH^Z), 8.47 (*s*, NH^{Aib}).
^{13}C-NMR (75.5 MHz, CD_3OD): 18.7, 19.7 (2 *q*, 2 γCH_3^{Val}), 24.4, 25.6 (2 *q*, 2 βCH_3^{Aib}), 26.6 (*t*, γCH_2^{Pro}), 28.8 (*t*, βCH_2^{Pro}), 32.0 (*d*, βCH^{Val}), 49.1 (*t*, δCH_2^{Pro}), 52.5 (*q*, CH_3^{OMe}), 57.4 (*s*, αC^{Aib}), 61.5 (*d*, αCH^{Val}), 62.0 (*d*, αCH^{Pro}), 67.5 (*t*, CH_2^Z), 128.7, 129.0, 129.5 (3 *d*, 5 arom. CH^Z), 138.3 (*s*, arom. C^Z), 158.5 (*s*, $OCONH^Z$), 173.3, 174.0, 174.9 (3 *s*, 3 CO).
ESI-MS (MeOH+NaI): 917.4 (20%, $[2M+Na]^+$), 469.9 (100%, $[M+Na]^+$).
Anal. calc. for $C_{23}H_{33}N_3O_6 \cdot 0.5\ H_2O$ (MG 456.53): C 60.51, H 7.51, N 9.20; found: C 60.70, H 8.33, N 9.46.
$[\alpha]_D^{23}$ = –42.1 (*c* = 1, AcOEt).

Synthesis of Z-Val-Aib-Pro-OH (68)

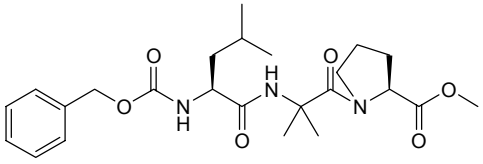


According to protocol C, the peptide methyl ester **61** (600 mg, 1.34 mmol) was dissolved in a mixture of THF, MeOH, and H_2O (40 ml), and $LiOH \cdot H_2O$ (169 mg, 4.02 mmol) was added. After the workup, the solvent was evaporated to give the C-unprotected tripeptide **68** (552 mg, 95%). White foam. R_f(A) = 0.04. M.p. 88-91 °C. RP-HPLC (II): t_R 33.94, λ 207.

IR (KBr): 3441*vs,* 3063*s,* 2966*vs,* 2877*s,* 1709*vs,* 1659*vs,* 1538*vs,* 1471*s,* 1455*vs,* 1423*vs,* 1366*s,* 1340*s,* 1291*s,* 1238*vs,* 1176*s,* 1096*m,* 1027*s,* 739*m,* 698*s,* 673*m,* 615*m*.
^{1}H-NMR (300 MHz, CD_3OD): 0.93, 0.97 (2 *d*, *J* = 6.7, 2 γCH_3^{Val}), 1.47 (*br*. *s*, 2 βCH_3^{Aib}), 1.56-1.62 (*m*, γCH_{2a}^{Pro}), 1.72-2.04 (*m*, βCH^{Val}, βCH_2^{Pro}, γCH_{2b}^{Pro}), 3.54-3.65 (*m*, δCH_2^{Pro}), 3.91 (*d*, *J* = 7.8, αCH^{Val}), 4.28-4.32 (*m*, αCH^{Pro}), 5.03, 5.14 (*AB*, *J* = 12.6, CH_2^Z), 7.28-7.36 (*m*, 5 arom. CH^Z).
^{13}C-NMR (75.5 MHz, CD_3OD): 18.7, 19.7 (2 *q*, 2 γCH_3^{Val}), 24.8, 25.6 (2 *q*, 2 βCH_3^{Aib}), 26.7 (*t*, γCH_2^{Pro}), 29.8 (*t*, βCH_2^{Pro}), 32.1 (*d*, βCH^{Val}), 49.1 (*t*, δCH_2^{Pro}), 57.9 (*s*, αC^{Aib}), 61.8 (*d*, αCH^{Val}), 64.8 (*d*, αCH^{Pro}), 67.6 (*d*, CH_2^Z), 128.8, 129.1, 129.5 (3 *d*, 5 arom. CH^Z). The quart. arom. C^Z, the carbamate and the CO's could not be detected.
ESI-MS (MeOH+NaI): 456.3 (100%, $[M+Na]^+$).

Anal. calc. for $C_{22}H_{31}N_3O_6 \cdot 0.5\ H_2O$ (MG 442.51): C 59.71, H 7.29, N 9.50; found: C 59.63, H 7.06, N 9.66.
$[\alpha]_D^{20} = -82.1$ (c = 1, MeOH).

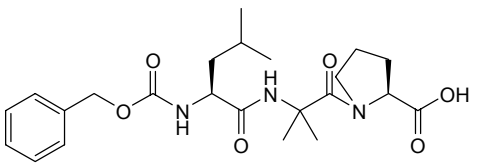
Synthesis of Z-Leu-Aib-Pro-OMe (62)



According to protocol B, the 2*H*-azirin-3-amine **2** (1.562 g, 7.969 mmol) was dissolved in CH_2Cl_2 (125 ml), Z-Leu-OH (1.762 g, 6.64 mmol) was added and the mixture stirred overnight. The solvent was evaporated and the product purified by flash chromatography to give the C-protected tripeptide **62** (3.031 g, 99%). White foam. R_f(A) = 0.65. M.p. 130-132 °C. RP-HPLC (II): t_R 22.55, λ 216.

IR (KBr): 3288*vs,* 3064*m,* 2954*s,* 2874*m,* 1753*vs,* 1714*vs,* 1666*vs,* 1623*vs,* 1536*vs,* 1414*vs,* 1384*s,* 1365*s,* 1267*vs,* 1240*vs,* 1205*s,* 1163*vs,* 1120*m,* 1043*s,* 1028*m,* 750*m,* 697*s,* 643*m*.
^{1}H-NMR (300 MHz, CD_3OD): 0.93, 0.96 (2 *d*, J = 6.3, 2 δCH_3^{Leu}), 1.42, 1.43 (2 *s*, 2 βCH_3^{Aib}), 1.45-1.61 (*m*, βCH_2^{Leu}), 1.62-1.81 (*m*, γCH^{Leu}, γCH_2^{Pro}), 1.81-1.98 (*m*, βCH_2^{Pro}), 3.53-3.67 (*m*, δCH_2^{Pro}), 3.67 (*s*, CH_3^{OMe}), 4.19 (*dd*, J = 9.9, 5.3, αCH^{Leu}), 4.38 (*dd*, J = 8.5, 3.6, αCH^{Pro}), 5.02, 5.14 (*AB*, J = 12.6, CH_2^Z), 7.28-7.36 (*m*, 5 arom. CH^Z).
^{13}C-NMR (75.5 MHz, CD_3OD): 21.8, 23.4 (2 *q*, 2 δCH_3^{Leu}), 24.4, 25.6 (2 *q*, 2 βCH_3^{Aib}), 25.9 (*d*, γCH^{Leu}), 26.7 (*t*, γCH_2^{Pro}), 28.8 (*t*, βCH_2^{Pro}), 42.0 (*t*, βCH_2^{Leu}), 49.2 (*t*, δCH_2^{Pro}), 52.4 (*q*, CH_3^{OMe}), 54.3 (*d*, αCH^{Leu}), 57.4 (*s*, αC^{Aib}), 62.1 (*d*, αCH^{Pro}), 67.5 (*t*, CH_2^Z), 128.6, 129.0, 129.5 (3 *d*, 5 arom. CH^Z), 138.5 (*s*, arom. C^Z), 158.4 (*s*, $OCONH^Z$), 174.0, 174.4, 174.8 (3 *s*, 3 CO).
ESI-MS (MeOH+NaI): 945.5 (5%, $[2M+Na]^+$), 484.1 (100%, $[M+Na]^+$).
Anal. calc. for $C_{24}H_{35}N_3O_6$ (MG 461.18): C 62.45, H 7.64, N 9.10; found: C 62.40, H 7.55, N 9.08.
$[\alpha]_D^{20} = -57.5$ (c = 1.25, AcOEt).

Synthesis of Z-Leu-Aib-Pro-OH (63)

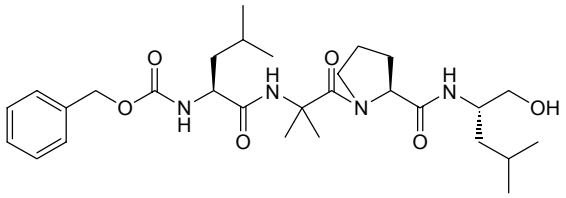


According to protocol C, the peptide methyl ester **62** (1.3 g, 2.82 mmol) was dissolved in a mixture of THF, MeOH, and H_2O (84 ml), and $LiOH \cdot H_2O$ (355 mg, 8.45 mmol) was added. After the workup, the solvent was evaporated to give the C-unprotected

tripeptide **63** (1.223 g, 97%). White foam. R_f(A) = 0.09. M.p. 82-86 °C. RP-HPLC (II): t_R 30.39, λ 215.

IR (KBr): 3323*vs,* 3066*s,* 2958*vs,* 1722*vs,* 1619*vs,* 1536*vs,* 1418*vs,* 1366*s,* 1341*s,* 1248*vs,* 1178*vs,* 1120*m,* 1045*s,* 741*m,* 698*s,* 638*m,* 608*m*.
^{1}H-NMR (300 MHz, CD_3OD): 0.93, 0.96 (2 *d*, *J* = 6.3, 2 δCH_3^{Leu}), 1.44 (*br. s*, 2 βCH_3^{Aib}), 1.45-1.61 (*m*, βCH_2^{Leu}), 1.62-1.76 (*m*, γCH^{Leu}), 1.76-1.85 (*m*, γCH_2^{Pro}), 1.85-1.99 (*m*, βCH_2^{Pro}), 3.55-3.66 (*m*, δCH_2^{Pro}), 4.19 (*dd*, *J* = 9.9, 5.3, αCH^{Leu}), 4.38 (*dd*, *J* = 8.4, 3.4, αCH^{Pro}), 5.02-5.14 (*AB*, *J* = 12.6, CH_2^Z), 7.28-7.36 (*m*, 5 arom. CH^Z).
^{13}C-NMR (75.5 MHz, CD_3OD): 21.8, 23.3 (2 *q*, 2 δCH_3^{Leu}), 24.4, 25.6 (2 *q*, 2 βCH_3^{Aib}), 25.8 (*d*, γCH^{Leu}), 26.6 (*t*, γCH_2^{Pro}), 29.0 (*t*, βCH_2^{Pro}), 42.0 (*t*, βCH_2^{Leu}), 49.1 (*t*, δCH_2^{Pro}), 54.3 (*d*, αCH^{Leu}), 57.4 (*s*, αC^{Aib}), 62.0 (*d*, αCH^{Pro}), 67.4 (*t*, CH_2^Z), 128.7, 129.0, 129.4 (5 *d*, 5 arom. CH^Z), 138.4 (*s*, arom. C^Z), 158.4 (*s*, $OCONH^Z$), 173.9, 174.4, 176.1 (3 *s*, 3 CO).
ESI-MS (MeOH+NaI): 470.2 (100%, $[M+Na]^+$).
Anal. calc. for $C_{23}H_{33}N_3O_6 \cdot H_2O$ (MG 462.52): C 59.34, H 7.58, N 9.03; found: C 59.49, H 7.38, N 9.37.
$[\alpha]_D^{20} = -80.9$ (*c* = 1, MeOH).

Synthesis of Z-Leu-Aib-Pro-Leuol (64)

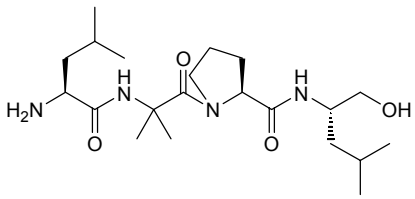


According to protocol A, the Z protected tripeptide **63** (1.223 g, 2.733 mmol), H-Leuol·HCl (419.9 mg, 2.733 mmol), HOBt (419.5 mg, 2.733 mmol), and TBTU (877.5 mg, 2.733 mmol) were dissolved in MeCN (170 ml), and TEA (1.15 ml, 8.199 mmol) was added. After the completion of the reaction, the mixture was purified by flash chromatography to give the tetrapeptide **64** (1.4 g, 94%). White powder. R_f(B) = 0.50. M.p. 60-63 °C. RP-HPLC (II): t_R 27.31, λ 207.

IR (KBr): 3292*vs,* 2957*vs,* 2871*m,* 1723*vs,* 1648*vs,* 1547*vs,* 1469*s,* 1412*s,* 1366*s,* 1253*s,* 1172*m,* 1044*s,* 740*m,* 698*m*.
^{1}H-NMR (300 MHz, CD_3OD): 0.86-0.98 (*m*, 2 δCH_3^{Leu}, 2 δCH_3^{Leuol}), 1.26-1.38 (*m*, βCH_{2a}^{Leu}, βCH_{2a}^{Leuol}), 1.40, 1.44 (2 *br. s*, 2 βCH_3^{Aib}), 1.47-1.68 (*m*, βCH_{2b}^{Leu}, βCH_{2b}^{Leuol}, γCH^{Leu}, γCH^{Leuol}), 1.68-1.82 (*m*, βCH_{2a}^{Pro}, γCH_2^{Pro}), 2.11-2.22 (*m*, βCH_{2b}^{Pro}), 3.37-3.61 (*m*, δCH_{2ab}^{Pro}), 3.52 (*d*, *J* = 5.7, CH_2OH^{Leuol}), 3.93-4.06 (*m*, αCH^{Leuol}), 4.13-4.21 (*m*, αCH^{Leu}), 4.37-4.45 (*m*, αCH^{Pro}), 5.05-5.17 (*AB*, *J* = 12.6, CH_2^Z), 7.29-7.43 (*m*, 5 H, arom. CH^Z).
^{13}C-NMR (75.5 MHz, CD_3OD): 21.9 (*q*, δCH_3^{Leu}), 22.3 (*q*, δCH_3^{Leuol}), 23.4(*q*, δCH_3^{Leu}), 24.0 (*q*, δCH_3^{Leuol}), 24.1 (*q*, βCH_3^{Aib}), 25.7 (*d*, γCH^{Leu}), 25.9 (*d*, γCH^{Leuol}), 26.1 (*q*, βCH_3^{Aib}), 26.5 (*t*, γCH_2^{Pro}), 29.9 (*t*, βCH_2^{Pro}), 40.4 (*t*, βCH_2^{Leuol}), 41.6 (*t*, βCH_2^{Leu}), 49.5 (*t*, δCH_2^{Pro}), 51.0 (*d*, αCH^{Leuol}), 55.1 (*d*, αCH^{Leu}), 57.7 (*s*, αC^{Aib}), 64.1 (*d*, αCH^{Pro}), 65.7

(*t*, CH_2OH^{Leuol}), 67.5 (*t*, CH_2^{Z}), 128.6, 128.9, 129.4 (3 *d*, 5 arom. CH^{Z}), 138.2 (*s*, arom. C^{Z}), 158.0 (*s*, $OCONH^{Z}$), 174.1, 174.3, 174.9 (3 *s*, 3 CO).
ESI-MS (MeOH+NaI): 569.5 (100%, $[M+Na]^+$), 547.4 (50%, $[M+H]^+$).
Anal. calc. for $C_{29}H_{46}N_4O_6$ (MG 546.23): C 63.71, H 8.48, N 10.25; found: C 63.32, H 8.40, N 10.19.
$[\alpha]_D^{20} = -31.2$ (*c* = 1, MeOH).

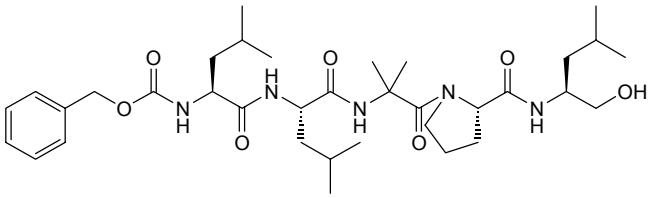
Synthesis of H-Leu-Aib-Pro-Leuol (65)



According to protocol D, the tetrapeptide **64** (447 mg, 0.818 mmol) was dissolved in MeOH (29 ml), Pd/C was added, and the mixture was stirred overnight under an H_2 atmosphere. After completion of the reaction and filtration through *Celite*, the solvent was evaporated leading to the N-unprotected tetrapeptide **65** (335 mg, quant.). Colorless oil. R_f(B) = 0.09. RP-HPLC (II): t_R 26.79, λ 203.

IR (KBr): 3292*vs,* 2956*vs,* 2871*s,* 1644*vs,* 1547*vs,* 1470*s,* 1411*s,* 1366*s,* 1196*m,* 1173*m*.
^{1}H-NMR (300 MHz, CD_3OD): 0.86-1.00 (*m*, 2 δCH_3^{Leu}, 2 δCH_3^{Leuol}), 1.27-1.43 (*m*, βCH_{2a}^{Leu}, βCH_{2a}^{Leuol}), 1.47, 1.48 (2 *s*, 2 βCH_3^{Aib}), 1.49-1.66 (*m*, βCH_{2b}^{Leuol}, βCH_{2b}^{Leu}, γCH^{Leuol}), 1.72-1.86 (*m*, γCH^{Leu}, βCH_{2a}^{Pro}), 1.86-1.97 (*m*, γCH_{2ab}^{Pro}), 2.20-2.34 (*m*, βCH_{2b}^{Pro}), 3.37-3.49 (*m*, δCH_{2a}^{Pro}, αCH^{Leu}), 3.53 (*d*, *J* = 5.3, CH_2OH^{Leuol}), 3.77-3.87 (*m*, δCH_{2b}^{Pro}), 3.94-4.05 (*m*, αCH^{Leuol}), 4.43 (*dd*, *J* = 8.4, 6.3, αCH^{Pro}).
^{13}C-NMR (75.5 MHz, CD_3OD): 22.3 (*q*, δCH_3^{Leu}, δCH_3^{Leuol}), 23.6 (*q*, δCH_3^{Leu}), 24.0(*q*, δCH_3^{Leuol}), 24.2 (*q*, βCH_3^{Aib}), 25.7 (*d*, γCH^{Leu}), 26.0 (*d*, γCH^{Leuol}), 26.4 (*q*, βCH_3^{Aib}), 26.6 (*t*, γCH_2^{Pro}), 30.0 (*t*, βCH_2^{Pro}), 40.5 (*t*, βCH_2^{Leuol}), 44.9 (*t*, βCH_2^{Leu}), 49.6 (*t*, δCH_2^{Pro}), 50.9 (*d*, αCH^{Leuol}), 54.0 (*d*, αCH^{Leu}), 57.5 (*s*, αC^{Aib}), 64.2 (*d*, αCH^{Pro}), 65.7 (*t*, CH_2OH^{Leuol}), 174.1, 174.5, 177.3 (3 *s*, 3 CO).
ESI-MS (MeOH+NaI): 435.3 (100%, $[M+Na]^+$).
$[\alpha]_D^{20} = -6.1$ (*c* = 1, MeOH).

Synthesis of Z-Leu-Leu-Aib-Pro-Leuol (66)

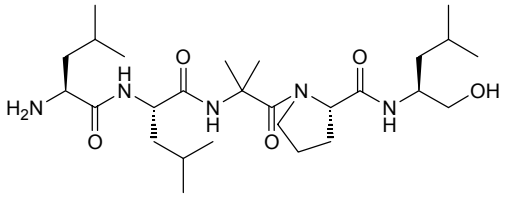


According to protocol A, to a mixture of Z-Leu-OH (513.7 mg, 1.936 mmol), the N-unprotected tetrapeptide **65** (789.8 mg, 1.936 mmol), HOBt (297.3 mg, 1.936 mmol),

and TBTU (621.7 mg, 1.936 mmol) dissolved in MeCN (122 ml), TEA (816 µl, 5.809 mmol) was added. After the completion of the reaction, the mixture was purified by flash chromatography to give the pentapeptide **66** (1.098 g, 86%). White powder. R_f(B) = 0.48. M.p. 88-90 °C. RP-HPLC (II): t_R 30.40, λ 210.

IR (KBr): 3416*s,* 3308*s,* 2957*vs,* 2871*m,* 1702*vs,* 1646*vs,* 1534*s,* 1469*s,* 1452*s,* 1421*s,* 1386*s,* 1366*s,* 1342*m,* 1310*m,* 1262*s,* 1222*m,* 1172*m,* 1151*m,* 1122*m,* 1083*s,* 1050*s,* 697*m*.
^{1}H-NMR (300 MHz, CD_3OD): 0.85-1.00 (*m*, 2 δCH_3^{Leu}, 2 δCH_3^{Leu}, 2 δCH_3^{Leuol}), 1.29-1.42 (*m*, βCH_{2a}^{Leu}, βCH_{2a}^{Leu}, βCH_{2a}^{Leuol}), 1.42, 1.45 (2 *s*, 2 βCH_3^{Aib}), 1.48-1.57 (*m*, βCH_{2b}^{Leu}, βCH_{2b}^{Leu}, βCH_{2b}^{Leuol}), 1.57-1.73 (*m*, γCH^{Leu}, γCH^{Leu}, γCH^{Leuol}), 1.74-1.85 (*m*, βCH_{2a}^{Pro}), 1.85-1.96 (*m*, γCH_2^{Pro}), 2.19-2.29 (*m*, βCH_{2b}^{Pro}), 3.43-3.56 (*m*, CH_2OH^{Leuol}, δCH_{2a}^{Pro}), 3.67-3.77 (*m*, δCH_{2b}^{Pro}), 3.94-4.03 (*m*, αCH^{Leuol}), 4.03-4.10 (*m*, αCH^{Leu}), 4.39-4.47 (*m*, αCH^{Leu}, αCH^{Pro}), 5.07-5.15 (*m*, CH_2^Z), 7.30-7.40 (*m*, 5 arom. CH^Z).
^{13}C-NMR (75.5 MHz, CD_3OD): 21.7, 22.1 (2 *q*, 2 δCH_3^{Leu}), 22.3 (*q*, 2 δCH_3^{Leuol}), 23.3, 23.5 (2 *q*, 2 δCH_3^{Leu}), 24.1, 24.3 (2 *q*, 2 βCH_3^{Aib}), 25.9, 26.1, 26.2 (3 *d*, γCH^{Leu}, γCH^{Leu}, γCH^{Leuol}), 26.7 (*t*, γCH_2^{Pro}), 30.1 (*t*, βCH_2^{Pro}), 40.7, 40.9, 41.9 (3 *t*, βCH_2^{Leu}, βCH_2^{Leu}, βCH_2^{Leuol}), 49.7 (*t*, δCH_2^{Pro}), 51.1 (*d*, αCH^{Leuol}), 52.9 (*d*, αCH^{Leu}), 55.8 (*d*, αCH^{Leu}), 57.9 (*s*, αC^{Aib}), 64.3 (*d*, αCH^{Pro}), 65.8 (*t*, CH_2OH^{Leuol}), 67.9 (*t*, CH_2^Z), 128.7, 129.1, 129.6 (3 *d*, 5 arom. CH^Z), 138.1 (*s*, arom. C^Z), 158.9 (*s*, $OCONH^Z$), 174.2, 174.4, 174.6, 175.7 (4 *s*, 4 CO).
ESI-MS (MeOH+NaI): 682.4 (100%, $[M+Na]^+$).
Anal. calc. for $C_{35}H_{57}N_5O_7 \cdot H_2O$ (MG 677.88): C 62.01, H 8.77, N 10.33; found: C 61.80, H 9.07, N 10.25.
$[\alpha]_D^{23} = -33.4$ (*c* = 1, AcOEt).

Synthesis of H-Leu-Leu-Aib-Pro-Leuol (67)

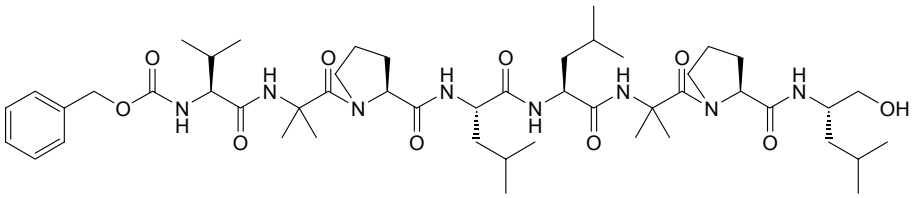


According to protocol D, the pentapeptide **66** (1.10 g, 1.667 mmol) was dissolved in MeOH (60 ml), Pd/C was added, and the mixture was stirred overnight under an H_2 atmosphere. After completion of the reaction and filtration through *Celite*, the solvent was evaporated leading to the N-unprotected pentapeptide **67** (856.2 mg, 98%). White foam. R_f(B) = 0.11. M.p. 45-50 °C. RP-HPLC (II): t_R 30.48, λ 210.

IR (KBr): 3302*vs,* 2957*vs,* 2871*s,* 1648*vs,* 1546*vs,* 1469*s,* 1455*s,* 1411*s,* 1385*s,* 1366*s,* 1341*m,* 1196*m,* 1172*m*.
^{1}H-NMR (300 MHz, CD_3OD): 0.90-0.98 (*m*, 2 δCH_3^{Leu}, 2 δCH_3^{Leu}, 2 δCH_3^{Leuol}), 1.28-1.55 (*m*, βCH_2^{Leu}, βCH_2^{Leuol}), 1.44 (*s*, 2 βCH_3^{Aib}), 1.55-1.74 (*m*, γCH^{Leu}, βCH_2^{Leu}, γCH_2^{Leu}, γCH^{Leuol}), 1.74-1.85 (*m*, βCH_{2a}^{Pro}), 1.85-1.95 (*m*, γCH_2^{Pro}), 2.19-2.29 (*m*,

βCH$_{2b}^{Pro}$), 3.39-3.58 (*m*, C*H*$_2$OH^{Leuol}, αCH^{Leu}, δCH$_{2a}^{Pro}$), 3.64-3.77 (*m*, δCH$_{2b}^{Pro}$), 3.95-4.03 (*m*, αCH^{Leuol}), 4.35-4.51 (*m*, αCH^{Pro}, αCH^{Leu}).
^{13}C-NMR (75.5 MHz, CD$_3$OD): 21.9, 22.0, 22.3, 22.5, 23.4, 23.6, 24.1, 24.2 (8 *q*, 2 δCH$_3^{Leu}$, 2 δCH$_3^{Leu}$, 2 δCH$_3^{Leuol}$, 2 βCH$_3^{Aib}$), 25.9, 26.1 (2 *d*, γCH^{Leu}, γCH^{Leu}, γCH^{Leuol}), 26.7 (*t*, γCH$_2^{Pro}$), 30.1 (*t*, βCH$_2^{Pro}$), 40.8, 41.9, 44.4 (3 *t*, βCH$_2^{Leu}$, βCH$_2^{Leu}$, βCH$_2^{Leuol}$), 49.7 (*t*, δCH$_2^{Pro}$), 51.2 (*d*, αCH^{Leuol}), 52.5 (*d*, αCH^{Leu}), 57.9 (*s*, αC^{Aib}), 59.3 (*d*, αCH^{Leu}), 64.2 (*d*, αCH^{Pro}), 65.8 (*t*, CH$_2$OH^{Leuol}), 174.3, 174.4, 174.5, 178.3 (4 *s*, 4 CO).
ESI-MS (MeOH+NaI): 548.5 (100%, [*M*+Na]$^+$), 526.5 (70%, [*M*+H]$^+$).
$[\alpha]_D^{20}$ = –50.3 (*c* = 1, MeOH).

Synthesis of Z-Val-Aib-Pro-Leu-Leu-Aib-Pro-Leuol (69)

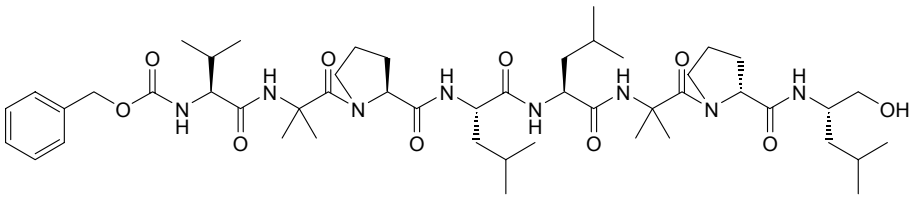


According to protocol A, to a mixture of the tripeptide Z-Val-Aib-Pro-OH **68** (38 mg, 0.0875 mmol), the N-unprotected pentapeptide **67** (46 mg, 0.0875 mmol), HOBt (13.4 mg, 0.0875 mmol), and TBTU (28.1 mg, 0.0875 mmol) dissolved in MeCN (6 ml), TEA (37 μl, 0.263 mmol) was added. After the completion of the reaction, the mixture was purified by flash chromatography to lead to the octapeptide **69** (45 mg, 55%). White powder. R_f(B) = 0.24. M.p. 125-130 °C. RP-HPLC (II): t_R *ca.* 100, λ 200.

IR (KBr): 3444*vs,* 2959*s,* 2873*m,* 1725*s,* 1644*vs,* 1540*vs,* 1470*s,* 1454*s,* 1416*vs,* 1366*m,* 1265*s,* 1233*m,* 1172*m,* 1027*m,* 699*m,* 619*m*.
^{1}H-NMR (500 MHz, CD$_3$OD): 0.84, 0.87 (2 *d*, *J* = 6.7, 2 δCH$_3^{Leuol}$), 0.89, 0.91 (2 *d*, *J* = 6.7, 2 δCH$_3^{Leu}$), 0.95-1.02 (*m*, 2 γCH$_3^{Val}$, 2 δCH$_3^{Leu}$), 1.31-1.37 (*m*, βCH$_{2a}^{Leuol}$), 1.45, 1.48 (2 *s*, 2 CH$_3^{Aib}$, 2 CH$_3^{Aib}$), 1.55-1.76 (*m*, βCH$_{2a}^{Pro}$, βCH$_{2b}^{Leuol}$, γCH^{Leuol}, γCH^{Leu}, βCH$_2^{Leu}$), 1.73-1.81 (*m*, γCH^{Leu}, βCH$_{2a}^{Pro}$), 1.82-1.90 (*m*, γCH$_2^{Pro}$, γCH$_{2a}^{Pro}$), 1.91-1.98 (*m*, γCH$_{2b}^{Pro}$, βCH$_2^{Leu}$), 2.14-2.21 (*m*, βCH^{Val}), 2.23-2.33 (*m*, βCH$_{2b}^{Pro}$, βCH$_{2b}^{Pro}$), 3.32-3.36 (*m*, δCH$_{2a}^{Pro}$), 3.46-3.60 (*m*, C*H*$_2$OH^{Leuol}, δCH$_2^{Pro}$), 3.80-3.85 (*m*, δCH$_{2b}^{Pro}$), 3.92 (*d*, *J* = 6.1, αCH^{Val}), 3.94-4.01 (*m*, αCH^{Leuol}), 4.16-4.20 (*m*, αCH^{Leu}), 4.31 (*dd*, *J* = 8.5, 6.7, αCH^{Pro}), 4.43-4.46 (*m*, αCH^{Pro}, αCH^{Leu}), 5.09, 5.20 (*AB*, *J* = 12.8, CH$_2^Z$), 7.32-7.41 (*m*, 5 arom. CH^Z).
^{13}C-NMR (125.7 MHz, CD$_3$OD): 19.9, 21.0 (2 *q*, 2 γCH$_3^{Val}$), 21.2, 22.1 (2 *q*, 2 δCH$_3^{Leu}$), 23.5, 23.6, 23.7, 24.2, 24.3 (5 *q*, 2 δCH$_3^{Leuol}$, 2 βCH$_3^{Aib}$, 2 δCH$_3^{Leu}$), 25.8 (*d*, γCH^{Leu}), 26.0 (*d*, γCH^{Leuol}), 26.1 (*q*, βCH$_3^{Aib}$), 26.1 (*d*, γCH^{Leu}), 26.7 (*t*, γCH$_2^{Pro}$), 26.8 (*q*, βCH$_3^{Aib}$), 27.0 (*t*, γCH$_2^{Pro}$), 29.9 (*t*, βCH$_2^{Pro}$), 30.2 (*t*, βCH$_2^{Pro}$), 31.5 (*d*, βCH^{Val}), 40.4 (2 *t*, βCH$_2^{Leu}$, βCH$_2^{Leuol}$), 41.3 (*t*, βCH$_2^{Leu}$), 49.9 (*t*, δCH$_2^{Pro}$), 50.3 (*t*, δCH$_2^{Pro}$), 51.2 (*d*, αCH^{Leuol}), 53.2 (*d*, αCH^{Leu}), 55.2 (*d*, αCH^{Leu}), 57.8 (*s*, αC^{Aib}), 57.9 (*s*, αC^{Aib}), 63.2 (*d*, αCH^{Val}), 64.3 (*d*, αCH^{Pro}), 65.1 (*d*, αCH^{Pro}), 65.8 (*t*, CH$_2$OH^{Leuol}), 67.9 (*t*, CH$_2^Z$), 128.9, 129.1, 129.5 (3 *d*, 5 arom. CH^Z), 138.3 (*s*, arom C^Z), 158.4 (*s*, OCONH^Z), 174.2, 174.3, 174.6, 174.9, 175.0, 175.9 (6 *s*, 7 CO).
ESI-MS (MeOH+NaI): 963.5 (100%, [*M*+Na]$^+$), 493.2 (15%, [*M*+2Na]$^{2+}$).

Anal. calc. for $C_{49}H_{80}N_8O_{10}$ (MG 940.39): C 62.53, H 8.57, N 11.91; found: C 62.37, H 8.40, N 11.64.
$[\alpha]_D^{20} = -7.2$ (c = 1, MeOH).

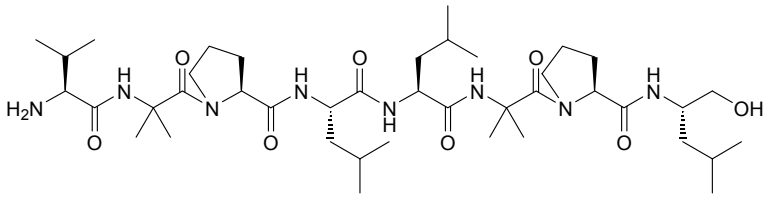
Z-Val-Aib-Pro-Leu-Leu-Aib-(*R*)Pro-Leuol (70)



After the purification of **69** by flash chromatography, a small fraction was isolated and identified as **70** (< 5 mg, see [1]) and [2])). White solid. R_f(B) = 0.40.

^{1}H-NMR (300 MHz, CD_3OD): due to the low amount of substance, the spectra quality of the spectra is low. But a visual comparison with the spectra of **70** shows substantial differences.
ESI-MS (MeCN, MeOH, H_2O): 964.0 (20%, $[M+Na]^+$), 727.6 (20%), 642.6 (10%), 529.3 (20%), 409.4 (100%).

Synthesis of H-Val-Aib-Pro-Leu-Leu-Aib-Pro-Leuol (71)

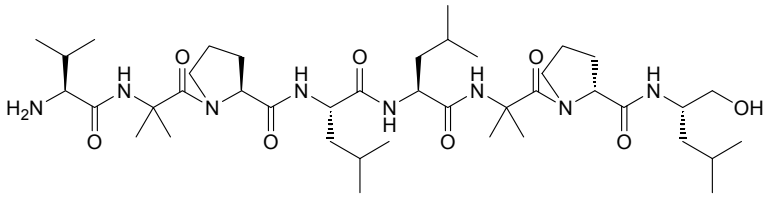


According to protocol D, the Z protected octapeptide **69** (533 mg, 0.566 mmol) was dissolved in MeOH (19 ml), Pd/C was added, and the mixture was stirred overnight under an H_2 atmosphere. After the completion of the reaction and filtration through *Celite*, the solvent was evaporated leading to the N-unprotected octapeptide **71** (388 mg, 85%). White foam. R_f(B) = 0.06. M.p. 110-115 °C. RP-HPLC (II): t_R 40.12, λ 202.

IR (KBr): 3302*vs,* 2958*vs,* 2872*m,* 1646*vs,* 1540*vs,* 1470*s,* 1413*s,* 1385*m,* 1365*m,* 1285*m,* 1196*m,* 1172*m,* 618*m*.
^{1}H-NMR (300 MHz, CD_3OD): 0.85 (*d*, *J* = 5.9, δCH_3^{Leuol}), 0.88-0.92 (*m*, δCH_3^{Leuol}, γCH_3^{Val}, δCH_3^{Leu}, δCH_3^{Leu}), 0.97 (*d*, *J* = 6.6, δCH_3^{Leu}), 0.98 (*d*, *J* = 6.6, δCH_3^{Leu}), 1.03 (*d*, *J* = 6.6, γCH_3^{Val}), 1.32-1.37 (*m*, βCH_{2a}^{Leuol}), 1.47, 1.49, 1.52, 1.55 (4 *s*, 2 βCH_3^{Aib}, 2 βCH_3^{Aib}), 1.57-1.63 (*m*, βCH_{2b}^{Leuol}), 1.64-1.77 (*m*, γCH^{Leu}, γCH^{Leu}, γCH^{Leuol}, βCH_2^{Leu}), 1.72-1.82 (*m*, βCH_{2a}^{Pro}, βCH_{2a}^{Pro}), 1.85-1.91 (*m*, γCH_{2a}^{Pro}), 1.92-1.98 (*m*, γCH_{2b}^{Pro}, βCH_2^{Leu}), 2.00 (*t*, *J* = 6.6, γCH_2^{Pro}), 2.17-2.22 (*m*, βCH^{Val}), 2.25-2.30 (*m*, βCH_{2b}^{Pro}), 2.35-2.40 (*m*, βCH_{2b}^{Pro}), 3.26 (*d*, *J* = 4.4, αCH^{Val}), 3.33-3.37 (*m*, δCH_{2a}^{Pro}), 3.46-3.57 (*m*, CH_2OH^{Leuol}, δCH_{2a}^{Pro}), 3.80-3.84 (*m*, δCH_{2b}^{Pro}), 3.90-3.94 (*m*, δCH_{2b}^{Pro}), 3.95-3.99

(*m*, αCH^{Leuol}), 4.19 (*dd*, *J* = 11.7, 4.4, αCH^{Leu}), 4.36 (*t*, *J* = 8.1, αCH^{Pro}), 4.43-4.47 (*m*, αCH^{Pro}, αCH^{Leu}).
^{13}C-NMR (75.5 MHz, CD_3OD): 17.2, 20.1 (2 *q*, 2 γCH$_3^{Val}$), 21.2, 21.4 (2 *q*, 2 δCH$_3^{Leuol}$), 22.3 (*q*, δCH$_3^{Leu}$), 23.7, 23.8 (2 *q*, δCH$_3^{Leu}$, δCH$_3^{Leu}$, βCH$_3^{Aib}$), 24.3 (*q*, δCH$_3^{Leu}$), 24.4 (*q*, βCH$_3^{Aib}$), 25.9 (*d*, γCH^{Leu}), 26.1 (*d*, γCH^{Leu}), 26.3 (*q*, βCH$_3^{Aib}$), 26.3 (*d*, γCH^{Leuol}), 26.8 (*t*, γCH$_2^{Pro}$), 27.2 (*q*, βCH$_3^{Aib}$), 27.2 (*d*, γCH$_2^{Pro}$), 30.1 (*t*, βCH$_2^{Pro}$), 30.2 (*t*, βCH$_2^{Pro}$), 33.0 (*d*, βCH^{Val}), 40.5 (*t*, βCH$_2^{Leu}$, βCH$_2^{Leuol}$), 41.4 (*t*, βCH$_2^{Leu}$), 50.1 (*t*, δCH$_2^{Pro}$), 50.3 (*t*, δCH$_2^{Pro}$), 51.2 (*d*, αCH^{Leuol}), 53.1 (*d*, αCH^{Leu}), 55.0 (*d*, αCH^{Leu}), 57.6 (*s*, αC^{Aib}), 57.9 (*s*, αC^{Aib}), 61.4 (*d*, αCH^{Val}), 64.3 (*d*, αCH^{Pro}), 65.2 (*d*, αCH^{Pro}), 65.9 (*t*, CH$_2$OH^{Leuol}), 174.2, 174.4, 174.6, 175.3, 175.8, 177.7 (6 *s*, 7 CO).
ESI-MS (MeOH+NaI): 829.6 (100%, [*M*+Na]$^+$), 426.3 (20%, [*M*+2Na]$^{2+}$).
Anal. calc. for $C_{41}H_{74}N_8O_8 \cdot 0.5\ H_2O$ (MG 816.09): C 60.34, H 9.26, N 13.73; found: C 60.10, H 9.29, N 13.35.
$[\alpha]_D^{23} = -2.7$ (*c* = 1.8, AcOEt).

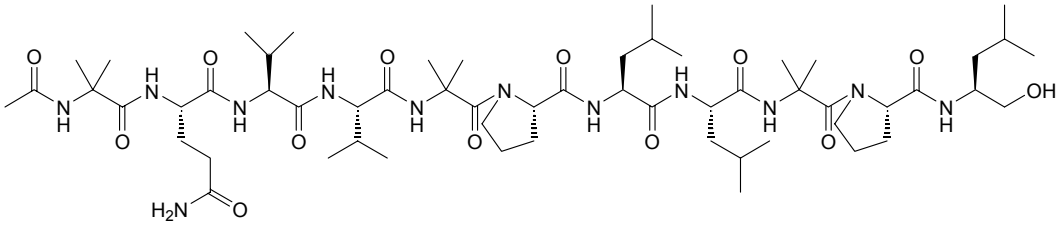
H-Val-Aib-Pro-Leu-Leu-Aib-(*R*)Pro-Leuol (72)



After the purification of **71** by flash chromatography, a small fraction was isolated and identified as **72** (< 10 mg, see [1]), [2]) and *Figure 24*). White crystals. R_f(B) = 0.24.

^{1}H-NMR (300 MHz, CD_3OD): 0.85 (*d*, *J* = 5.4, δCH$_3^{Leuol}$), 0.88-1.00 (*m*, δCH$_3^{Leuol}$, γCH$_3^{Val}$, δCH$_3^{Leu}$, δCH$_3^{Leu}$, δCH$_3^{Leu}$, δCH$_3^{Leu}$, γCH$_3^{Val}$), 1.32-1.39 (*m*, βCH$_{2a}^{Leuol}$), 1.44, 1.45, 1.46, 1.47 (4 *s*, 2 βCH$_3^{Aib}$, 2 βCH$_3^{Aib}$), 1.53-1.85 (*m*, βCH$_{2b}^{Leuol}$, γCH^{Leu}, γCH^{Leu}, γCH^{Leuol}, βCH$_2^{Leu}$, βCH$_{2a}^{Pro}$, βCH$_{2a}^{Pro}$), 1.85-1.99 (*m*, γCH$_{2a}^{Pro}$, γCH$_{2b}^{Pro}$, βCH$_2^{Leu}$), 1.99-2.01 (*m*, γCH$_2^{Pro}$), 2.17-2.34 (*m*, βCH^{Val}, βCH$_{2b}^{Pro}$), 2.35-2.43 (*m*, βCH$_{2b}^{Pro}$), 3.34-3.44 (*m*, δCH$_{2a}^{Pro}$, αCH^{Val}), 3.44-3.59 (*m*, C*H*$_2$OH^{Leuol}, δCH$_{2a}^{Pro}$), 3.63-3.76 (*m*, δCH$_{2b}^{Pro}$), 3.76-3.89 (*m*, δCH$_{2b}^{Pro}$), 3.89-4.02 (*m*, αCH^{Leuol}), 4.18-4.26 (*m*, αCH^{Leu}), 4.26-4.38 (*m*, αCH^{Pro}), 4.39-4.48 (*m*, αCH^{Pro}, αCH^{Leu}).

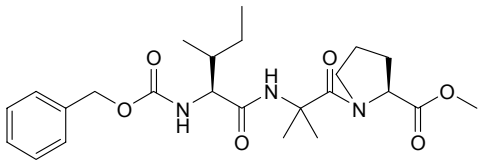
Synthesis of *Hypomurocin A1*, Ac-Aib-Gln-Val-Val-Aib-Pro-Leu-Leu-Aib-Pro-Leuol (5)



According to protocol A, the tripeptide Ac-Aib-Gln-Val-OH (**51**; 153.7 mg, 0.413 mmol), the N-unprotected octapeptide **71** (333 mg, 0.413 mmol), HOBt (63.3 mg, 0.413 mmol), and TBTU (132.5 mg, 0.413 mmol) were dissolved in MeCN (27 ml), and TEA (174 µl, 1.238 mmol) was added. After the completion of the reaction, the mixture was purified by flash chromatography to give the undecapeptaibol **5** (197 mg, 41%). White powder. R_f(C) = 0.35. M.p. 163-165 °C. RP-HPLC (II): t_R 51.38, λ 215.

IR (KBr): 3309*vs,* 2960*s,* 2873*m,* 1647*vs,* 1535*vs,* 1470*s,* 1414*s,* 1385*m,* 1366*m,* 1287*m,* 1195*m,* 1172*m,* 619*m*.
^{1}H-NMR (600 MHz, CD_3OD): 0.84 (*d*, *J* = 6.6, δCH_3^{Leu}), 0.88 (*d*, *J* = 6.6, δCH_3^{Leu}), 0.90 (*d*, *J* = 6.6, δCH_3^{Leuol}), 0.92 (*d*, *J* = 6.6, δCH_3^{Leu}), 0.97 (*d*, *J* = 6.6, γCH_3^{Val}), 0.99 (*d*, *J* = 6.6, δCH_3^{Leuol}), 1.00 (*d*, *J* = 6.6, δCH_3^{Leu}), 1.02 (2 *d*, *J* = 6.6, γCH_3^{Val}, γCH_3^{Val}), 1.07 (*d*, *J* = 6.6, γCH_3^{Val}), 1.33-1.37 (*m*, βCH_{2b}^{Leuol}), 1.46 (3 *s*, 2 βCH_3^{Aib}, βCH_3^{Aib}), 1.49 (*s*, βCH_3^{Aib}), 1.52 (*s*, βCH_3^{Aib}), 1.55 (*s*, βCH_3^{Aib}), 1.56-1.61 (*m*, βCH_{2b}^{Leu}, βCH_{2a}^{Leuol}), 1.62-1.81 (*m*, βCH_{2b}^{Pro}, γCH^{Leu}, βCH_{2b}^{Pro}, γCH^{Leuol}, βCH_{2ab}^{Leu}), 1.82-1.91 (*m*, γCH^{Leu}, γCH_{2b}^{Pro}), 1.95-2.02 (*m*, βCH_{2a}^{Leu}, γCH_{2b}^{Pro}, γCH_{2a}^{Pro}), 2.04 (*s*, CH_3^{Ac}), 2.05-2.15 (*m*, γCH_{2a}^{Pro}, βCH_2^{Gln}), 2.17-2.23 (*m*, βCH^{Val}), 2.25-2.32 (*m*, βCH_{2a}^{Pro}, βCH^{Val}), 2.33-2.36 (*m*, βCH_{2a}^{Pro}), 2.38-2.43 (*m*, γCH_{2b}^{Gln}), 2.46-2.50 (*m*, γCH_{2a}^{Gln}), 3.32-3.36 (*m*, δCH_{2b}^{Pro}), 3.49, 3.54 (*ABX*, *J* = 11.0, 5.9, CH_2OH^{Leuol}), 3.52-3.56 (*m*, δCH_{2b}^{Pro}), 3.80-3.86 (*m*, δCH_{2a}^{Pro}, δCH_{2a}^{Pro}), 3.94-3.98 (*m*, αCH^{Leuol}), 3.96 (*d*, *J* = 8.1, αCH^{Val}), 4.06 (*dd*, *J* = 7.3, 4.4, αCH^{Gln}), 4.11 (*d*, *J* = 7.3, αCH^{Val}), 4.17 (*dd*, *J* = 11.7, 3.7, αCH^{Leu}), 4.33 (*t*, *J* = 8.1, αCH^{Pro}), 4.43-4.48 (*m*, αCH^{Pro}, αCH^{Leu}).
^{13}C-NMR (150.9 MHz, CD_3OD): 19.6, 19.7 (2 *q*, γCH_3^{Val}, γCH_3^{Val}), 20.1 (*q*, γCH_3^{Val}, γCH_3^{Val}), 21.0 (*q*, δCH_3^{Leu}), 21.2 (*q*, δCH_3^{Leu}), 22.1 (*q*, δCH_3^{Leuol}), 23.2 (*q*, CH_3^{Ac}), 23.6 (*q*, δCH_3^{Leu}), 23.7 (*q*, δCH_3^{Leu}), 23.8 (*q*, βCH_3^{Aib}), 24.2 (*q*, δCH_3^{Leuol}, βCH_3^{Aib}), 24.3 (*q*, βCH_3^{Aib}), 25.9 (*d*, γCH^{Leu}), 26.1 (*q*, βCH_3^{Aib}), 26.1 (*d*, γCH^{Leuol}), 26.2 (*d*, γCH^{Leu}), 26.5 (*t*, βCH_2^{Gln}), 26.8 (*t*, γCH_2^{Pro}), 26.8 (*q*, βCH_3^{Aib}), 27.0 (*q*, βCH_3^{Aib}), 27.1 (*t*, γCH_2^{Pro}), 30.1 (*t*, βCH_2^{Pro}), 30.2 (*t*, βCH_2^{Pro}), 30.7 (*d*, βCH^{Val}), 31.5 (*d*, βCH^{Val}), 32.3 (*t*, γCH_2^{Gln}), 40.4 (*t*, βCH_2^{Leuol}), 40.5 (*t*, βCH_2^{Leu}), 41.4 (*t*, βCH_2^{Leu}), 50.4 (*t*, δCH_2^{Pro}), 50.7 (*t*, δCH_2^{Pro}), 51.2 (*d*, αCH^{Leuol}), 53.1 (*d*, αCH^{Leu}), 55.1 (*d*, αCH^{Leu}), 57.5, 57.9, 58.0 (3 *s*, αC^{Aib}, αC^{Aib}, αC^{Aib}), 57.6 (*d*, αCH^{Gln}), 61.6 (*d*, αCH^{Val}), 63.0 (*d*, αCH^{Val}), 64.4 (*d*, αCH^{Pro}), 65.2 (*d*, αCH^{Pro}), 65.8 (*t*, CH_2OH^{Leuol}), 173.8, 174.3, 174.4, 174.6, 174.7, 174.8, 175.1, 175.2, 176.1, 178.0, 178.5 (11 *s*, 12 CO).
ESI-MS (MeOH+NaI): 1184.1 (27%, $[M+Na]^+$), 603.7 (100%, $[M+2Na]^{2+}$).
Anal. calc. for $C_{57}H_{100}N_{12}O_{13} \cdot 2.5\ H_2O$ (MG 1206.53): C 56.74, H 8.77, N 13.93; found: C 56.63, H 8.57, N 13.73.
$[\alpha]_D^{23}$ = +3.3 (*c* = 0.9, AcOEt).

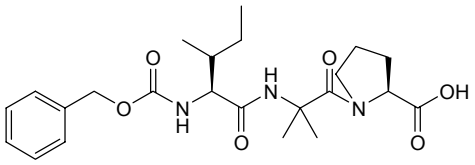
Synthesis of Z-Ile-Aib-Pro-OMe (74)



According to protocol B, **2** (1782.4 mg, 7.266 mmol) was dissolved in CH_2Cl_2 (80 ml), Z-Ile-OH (1752.5 mg, 6.605 mmol) was added, and the mixture was stirred overnight. Then, the solvent was evaporated and the crude product purified by flash chromatography to give **74** (1559 mg, 51%). White foam. R_f(A) = 0.65. M.p. 115-117 °C. RP-HPLC (II): λ 219.

IR (KBr): 3279*vs*, 3065*s,* 3036*m,* 2966*vs*, 2928*s,* 2877*m,* 1752*vs,* 1718*vs*, 1666*vs*, 1622*vs*, 1540*vs*, 1454*vs*, 1416*vs*, 1384*s,* 1364*vs*, 1271*vs*, 1241*vs*, 1205*vs*, 1162*vs*, 1125*s,* 1095*m,* 1046*s,* 1027*s,* 947*w,* 924*w,* 912*w,* 852*w,* 777*m,* 754 *m,* 739*s,* 697*s,* 646*m,* 616*m*.
^{1}H-NMR (300 MHz, CD_3OD): 0.86-0.97 (*m*, γCH_3^{Ile}, δCH_3^{Ile}), 1.13-1.30 (*m*, γCH_{2a}^{Ile}), 1.43, 1.45 (2 *s*, 2 βCH_3^{Aib}), 1.47-1.57 (*m*, γCH_{2b}^{Ile}), 1.58-1.68 (*m*, γCH_{2a}^{Pro}), 1.69-1.80 (*m*, βCH_{2b}^{Pro}, βCH^{Ile}), 1.80-1.95 (*m*, γCH_{2b}^{Pro}, βCH_{2a}^{Pro}), 3.46-3.57 (*m*, δCH_{2a}^{Pro}), 3.57-3.65 (*m*, δCH_{2b}^{Pro}), 3.67 (*s*, OMe), 3.93-4.00 (*m*, αCH^{Ile}), 4.38 (*dd*, *J* = 8.4, 3.7, αCH^{Pro}), 5.02, 5.14 (*AB*, *J* = 12.6, CH_2^Z), 7.07 (*br. d*, *J* = 8.8, NH^{Ile}), 7.25-7.38 (*m*, 5 arom. CH^Z), 8.49 (*br. s*, NH^{Aib}).
^{13}C-NMR (75.5 MHz, CD_3OD): 11.1 (*q*, δCH_3^{Ile}), 15.9 (*q*, γCH_3^{Ile}), 24.5 (*q*, βCH_3^{Aib}), 25.7 (*q*, βCH_3^{Aib}), 25.9 (*t*, γCH_2^{Ile}), 26.7 (*t*, γCH_2^{Pro}), 28.8 (*t*, βCH_2^{Pro}), 38.2 (*d*, βCH^{Ile}), 49.1 (*t*, δCH_2^{Pro}), 52.5 (*q*, OMe), 57.6 (*s*, αC^{Aib}), 60.5 (*d*, αCH^{Ile}), 62.0 (*d*, αCH^{Pro}), 67.5 (*t*, CH_2^Z), 128.7, 129.0, 129.5 (3 *d*, 5 arom. CH^Z), 138.4 (*s*, arom. C^Z), 158.4 (*s*, $OCONH^Z$), 173.5, 173.9, 174.8 (3 *s*, 3 CO).
ESI-MS (MeOH+NaI): 498.2 (20%), 484.2 (100%, $[M+Na]^+$).
Anal. calc. for $C_{24}H_{35}N_3O_6 \cdot 0.5\ H_2O$ (MG 470.51): C 61.27, H 7.71, N 8.93; found: C 61.33, H 7.89, N 8.87.
$[\alpha]_D^{20} = -69.0$ (*c* = 0.10, MeOH).

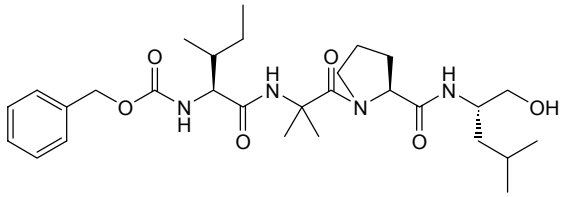
Synthesis of Z-Ile-Aib-Pro-OH (75)



According to protocol C, **74** (1.71 g, 3.70 mmol) was dissolved in a mixture of THF, MeOH, and H_2O (126 ml), and $LiOH \cdot H_2O$ (466 mg, 11.11 mmol) was added. After the workup, the solvent was evaporated to give **75** (1.32 g, 80%). White foam. R_f(A) = 0.09. M.p. 90-92 °C. RP-HPLC (II): t_R 29.22, λ 218.

IR (KBr): 3417*vs,* 3036*s,* 2965*vs,* 2933*vs,* 2877*s,* 1713*vs,* 1611*vs,* 1535*vs,* 1455*vs,* 1403*vs,* 1251*vs,* 1175*s,* 1130*s,* 1089*s,* 1042*vs,* 1027*s,* 983*m,* 911*w,* 880*w,* 850*w,* 821*w,* 776*m,* 737*s,* 697*s,* 661*m,* 619*m.*
^{1}H-NMR (300 MHz, CD_3OD): 0.86-0.95 (*m*, γCH_3^{Ile}, δCH_3^{Ile}), 1.06-1.23 (*m*, γCH_{2a}^{Ile}), 1.44, 1.46 (2 *s*, 2 βCH_3^{Aib}), 1.50-1.59 (*m*, γCH_{2b}^{Ile}), 1.59-1.68 (*m*, γCH_{2a}^{Pro}), 1.69-1.95 (*m*, γCH_{2b}^{Pro}, βCH_2^{Pro}, βCH^{Ile}), 3.47-3.66 (*m*, δCH_2^{Pro}), 3.97 (*t*, *J* = 8.3, αCH^{Ile}), 4.38 (*dd*, *J* = 8.4, 3.5, αCH^{Pro}), 5.02, 5.15 (*AB*, *J* = 12.5, CH_2^Z), 7.06 (*br. d*, *J* = 8.8, NH^{Ile}), 7.26-7.37 (*m*, 5 arom. CH^Z), 8.49 (*br. s*, NH^{Aib}).
^{13}C-NMR (75.5 MHz, CD_3OD): 11.2 (*q*, δCH_3^{Ile}), 15.9 (*q*, γCH_3^{Ile}), 24.6 (*q*, βCH_3^{Aib}), 25.7 (*q*, βCH_3^{Aib}), 26.0 (*t*, γCH_2^{Ile}), 26.7 (*t*, γCH_2^{Pro}), 29.1 (*t*, βCH_2^{Pro}), 38.2 (*d*, βCH^{Ile}), 49.1 (*t*, δCH_2^{Pro}), 57.6 (*s*, αC^{Aib}), 60.5 (*d*, αCH^{Ile}), 62.0 (*d*, αCH^{Pro}), 67.5 (*t*, CH_2^Z), 128.7, 129.0, 129.5 (3 *d*, 5 arom. CH^Z), 138.4 (*s*, arom. C^Z), 158.5 (*s*, $OCONH^Z$), 173.5, 173.9, 176.1 (3 *s*, 3 CO).
ESI-MS (MeOH+NaI): 470.1 (100%, $[M+Na]^+$).
$[\alpha]_D^{20} = -48.6$ (*c* = 1.00, MeOH).

Synthesis of Z-Ile-Aib-Pro-Leuol (76)

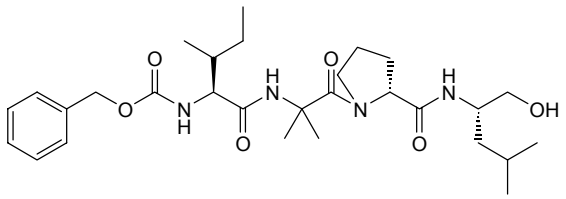


According to protocol A, a mixture of **75** (1.237 g, 2.76 mmol), H-Leuol · HCl (424.7 mg, 2.76 mmol), TBTU (887.5 mg, 2.76 mmol), and HOBt (373 mg, 2.76 mmol) was dissolved in MeCN (160 ml), and TEA (1166 µl, 8.29 mmol) was added. After completion of the reaction, the mixture was purified by flash chromatography to give **76** (946.5 mg, 63%). White solid. R_f(B) = 0.57. M.p. 192-195 °C. RP-HPLC (II): t_R 49.53, λ 219.

IR (KBr): 3466*vs,* 3392*s,* 3294*vs,* 3005*s,* 2972*vs,* 2877*s,* 1720*vs,* 1630*vs,* 1555*vs,* 1514*vs,* 1469*s,* 1413*vs,* 1381*s,* 1363*m,* 1327*s,* 1310*m,* 1286*s,* 1245*vs,* 1198*s,* 1171*s,* 1150*m,* 1087*s,* 1067*s,* 1037*s,* 1009*m,* 992*m,* 914*w,* 867*w,* 792*w,* 758*s,* 721*m,* 701*m,* 651*w,* 625*m.*
^{1}H-NMR (300 MHz, CD_3OD): 0.86-0.96 (*m*, 2 δCH_3^{Leuol}, γCH_3^{Ile}, δCH_3^{Ile}), 1.10-1.38 (*m*, βCH_{2a}^{Leuol}, γCH_{2a}^{Ile}), 1.41, 1.47 (2 br. *s*, 2 βCH_3^{Aib}), 1.50-1.85 (*m*, βCH_{2b}^{Leuol}, γCH_{2b}^{Ile}, γCH^{Leuol}, γCH_2^{Pro}, βCH_{2a}^{Pro}, βCH^{Ile}), 2.10-2.20 (*m*, βCH_{2b}^{Pro}), 3.33-3.43 (*m*, δCH_{2a}^{Pro}), 3.46 (*d*, *J* = 5.3, CH_2OH^{Leuol}), 3.63-3.71 (*m*, δCH_{2b}^{Pro}), 3.90-4.00 (*m*, αCH^{Leuol}), 4.01 (*d*, *J* = 8.1, αCH^{Ile}), 4.40 (*dd*, *J* = 8.5, 5.3, αCH^{Pro}), 5.03, 5.13 (*AB*, *J* = 12.7, CH_2^Z), 7.26-7.38 (*m*, 5 arom. CH^Z).
^{13}C-NMR (75.5 MHz, CD_3OD): 11.6 (*q*, δCH_3^{Ile}), 16.3 (*q*, γCH_3^{Ile}), 22.2, 23.9 (2 *q*, 2 δCH_3^{Leuol}), 24.3, 26.0 (2 *q*, 2 βCH_3^{Aib}), 26.1 (*t*, γCH_2^{Pro}), 26.1 (*d*, γCH^{Leuol}), 26.6 (*t*, γCH_2^{Ile}), 30.0 (*t*, βCH_2^{Pro}), 38.0 (*d*, βCH^{Ile}), 40.7 (*t*, βCH_2^{Leuol}), 49.6 (*t*, δCH_2^{Pro}), 51.2 (*d*, αCH^{Leuol}), 57.9 (*s*, αC^{Aib}), 61.4 (*d*, αCH^{Ile}), 64.2 (*d*, αCH^{Pro}), 65.8 (*t*, CH_2OH^{Leuol}),

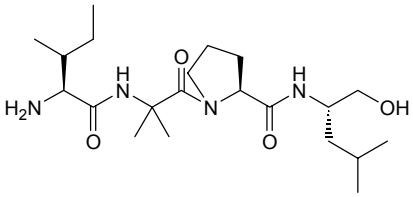
67.7 (*t*, CH_2^Z), 128.8, 129.0, 129.5 (3 *d*, 5 arom. CH^Z), 138.3 (*s*, arom. C^Z), 158.4 (*s*, $OCONH^Z$), 174.1, 174.5, 174.6 (3 *s*, 3 CO).
ESI-MS (MeOH+NaI): 569.3 (100%, $[M+Na]^+$).
Anal. calc. for $C_{29}H_{46}N_4O_6 \cdot H_2O$ (MG 564.72): C 61.68, H 8.57, N 9.92; found: C 61.98, H 8.21, N 9.99.
$[\alpha]_D^{20} = -28.7$ (*c* = 1.00, MeOH).

Z-Ile-Aib-(*R*)Pro-Leuol (79)



After the purification of **76** by flash chromatography, a small fraction was isolated and identified as **79** (~10 mg, ~1%, see *Figure 26*). White crystals.

Synthesis of H-Ile-Aib-Pro-Leuol (77)

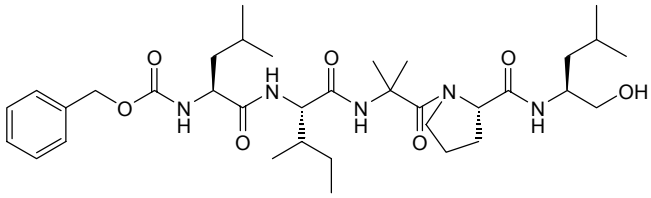


According to protocol D, to a solution of **76** (946.5 mg, 1.731 mmol) in MeOH (67 ml), Pd/C was added and the mixture was stirred under an H_2 atmosphere overnight. After completion of the reaction and filtration through *Celite*, the solvent was evaporated to give **77** (738 mg, quant.). White foam. R_f(B) = 0.13. M.p. 140-141 °C. RP-HPLC (II): t_R 25.02, λ 218.

IR (KBr): 3406*vs,* 3360*s,* 3278*vs,* 3071*w,* 2958*s,* 2933*s,* 2875*m,* 1639*vs,* 1548*s,* 1504*m,* 1465*s,* 1453*m,* 1406*s,* 1380*m,* 1361*m,* 1305*w,* 1279*w,* 1236*w,* 1195*w,* 1173*m,* 1149*m,* 1093*w,* 1073*w,* 1048*w,* 1033*w,* 708*w,* 618*w.*
^{1}H-NMR (300 MHz, CD_3OD): 0.88-0.96 (*m*, 2 δCH_3^{Leuol}, δCH_3^{Ile}), 1.05 (*d*, *J* = 6.8, γCH_3^{Ile}), 1.14-1.28 (*m*, γCH_{2a}^{Ile}), 1.29-1.40 (*m*, βCH_{2a}^{Leuol}), 1.48, 1.49 (2 *s*, 2 βCH_3^{Aib}), 1.45-1.57 (*m*, βCH_{2b}^{Leuol}, γCH_{2b}^{Ile}, γCH^{Leuol}), 1.76-1.97 (*m*, γCH_2^{Pro}, βCH_{2a}^{Pro}, βCH^{Ile}), 2.18-2.31 (*m*, βCH_{2b}^{Pro}), 3.38 (*d*, *J* = 4.2, αCH^{Ile}), 3.46-3.56 (*m*, CH_2OH^{Leuol}, δCH_{2b}^{Pro}), 3.72-3.82 (*m*, δCH_{2a}^{Pro}), 3.94-4.04 (*m*, αCH^{Leuol}), 4.43 (*dd*, *J* = 8.2, 5.8, αCH^{Pro}).
^{13}C-NMR (75.5 MHz, CD_3OD): 12.1 (*q*, δCH_3^{Ile}), 16.3 (*q*, γCH_3^{Ile}), 22.2, 23.8 (2 *q*, 2 δCH_3^{Leuol}), 24.3 (*q*, βCH_3^{Aib}), 25.1 (*t*, γCH_2^{Pro}), 26.1 (*d*, γCH^{Leuol}), 26.3 (*q*, βCH_3^{Aib}), 26.7 (*t*, γCH_2^{Ile}), 30.1 (*t*, βCH_2^{Pro}), 39.8 (*d*, βCH^{Ile}), 40.7 (*t*, βCH_2^{Leuol}), 49.6 (*t*, δCH_2^{Pro}), 50.1 (*d*, αCH^{Leuol}), 57.8 (*s*, αC^{Aib}), 60.3 (*d*, αCH^{Ile}), 64.3 (*d*, αCH^{Pro}), 65.8 (*t*, CH_2OH^{Leuol}), 174.4, 174.7, 175.4 (3 *s*, 3 CO).

ESI-MS (MeOH+NaI): 435.3 (100%, $[M+Na]^+$).
Anal. calc. for $C_{21}H_{40}N_4O_4 \cdot 0.5\ H_2O$ (MG 421.58): C 59.83, H 9.80, N 13.29; found: C 60.13, H 9.96, N 13.17.
$[\alpha]_D^{20} = -5.2$ ($c = 0.50$, MeOH).

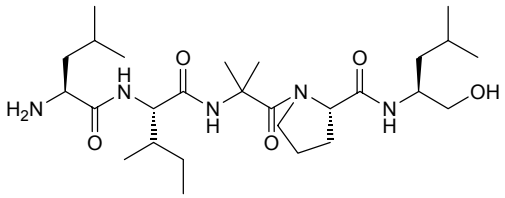
Synthesis of Z-Leu-Ile-Aib-Pro-Leuol (78)



According to protocol A, a mixture of **77** (700 mg, 1.70 mmol), Z-Leu-OH (450.1 mg, 1.70 mmol), TBTU (544.8 mg, 1.70 mmol), and HOBt (260.5 mg, 1.70 mmol) was dissolved in MeCN (70 ml), and TEA (715 µl, 5.09 mmol) was added. As **77** was not very soluble in MeCN, 20 ml DMF were added. After completion of the reaction, the mixture was purified by flash chromatography to give **78** (1086 mg, 97%). White foam. R_f(B) = 0.70. M.p. 88-90 °C. RP-HPLC (II): t_R 29.18, λ 218.

IR (KBr): 3308*vs,* 3035*m,* 2960*vs,* 2874*s,* 1704*vs,* 1648*s,* 1544*vs,* 1468*s,* 1454*s,* 1410*s,* 1384*s,* 1366*s,* 1342*m,* 1263*s,* 1172*m,* 1120*m,* 1043*s,* 739*w,* 697*m,* 616*m.*
^{1}H-NMR (300 MHz, CD_3OD): 0.86-0.98 (*m*, 2 δCH_3^{Leu}, 2 δCH_3^{Leuol}, δCH_3^{Ile}, γCH_3^{Ile}), 1.10-1.26 (*m*, γCH_{2a}^{Ile}), 1.28-1.39 (*m*, βCH_{2a}^{Leuol}), 1.40, 1.43 (2 *s*, 2 βCH_3^{Aib}), 1.44-1.64 (*m*, βCH_2^{Leu}, βCH_{2b}^{Leuol}, γCH_{2b}^{Ile}, γCH^{Leuol}), 1.64-1.77 (*m*, γCH^{Leu}), 1.77-1.85 (*m*, βCH_{2a}^{Pro}), 1.80-1.97 (*m*, γCH_2^{Pro}, βCH^{Ile}), 2.14-2.25 (*m*, βCH_{2b}^{Pro}), 3.51 (*t*, *J* = 5.6, CH_2OH^{Leuol}), 3.56-3.64 (*m*, δCH_2^{Pro}), 3.94-4.04 (*m*, αCH^{Leuol}), 4.12-4.18 (*m*, αCH^{Leu}), 4.23 (*d*, *J* = 7.2, αCH^{Ile}), 4.39-4.45 (*m*, αCH^{Pro}), 5.09, 5.16 (*AB*, *J* = 12.7, CH_2^Z), 7.26-7.38 (*m*, 5 arom. CH^Z).
^{13}C-NMR (75.5 MHz, CD_3OD): 11.5 (*q*, δCH_3^{Ile}), 16.3 (*q*, γCH_3^{Ile}), 21.9 (*q*, δCH_3^{Leu}), 22.2 (*q*, δCH_3^{Leuol}), 23.4 (*q*, δCH_3^{Leu}), 23.8 (*q*, δCH_3^{Leuol}), 24.2 (*q*, βCH_3^{Aib}), 25.8 (*q*, βCH_3^{Aib}), 25.9 (*t*, γCH_2^{Pro}), 26.0 (*d*, γCH^{Leuol}), 26.1 (*d*, γCH^{Leu}), 26.7 (*t*, γCH_2^{Ile}), 30.1 (*t*, βCH_2^{Pro}), 37.5 (*d*, βCH^{Ile}), 40.7 (*t*, βCH_2^{Leuol}), 41.9 (*t*, βCH_2^{Leu}), 49.3 (*t*, δCH_2^{Pro}), 51.0 (*d*, αCH^{Leuol}), 55.4 (*d*, αCH^{Ile}), 58.0 (*s*, αC^{Aib}), 59.0 (*d*, αCH^{Leu}), 64.2 (*d*, αCH^{Pro}), 65.8 (*t*, CH_2OH^{Leuol}), 67.7 (*t*, CH_2^Z), 128.7, 129.1, 129.6 (3 *d*, 5 arom. CH^Z), 138.3 (*s*, arom. C^Z), 158.6 (*s*, $OCONH^Z$), 173.1, 174.4, 174.6, 175.4 (4 *s*, 4 CO).
ESI-MS (MeOH+NaI): 682.3 (44%, $[M+Na]^+$), 435.3 (100%).
Anal. calc. for $C_{35}H_{57}N_5O_7 \cdot 0.5\ H_2O$ (MG 668.41): C 62.89, H 8.75, N 10.48; found: C 62.62, H 8.91, N 10.54.
$[\alpha]_D^{20} = -56.9$ ($c = 1.00$, MeOH).

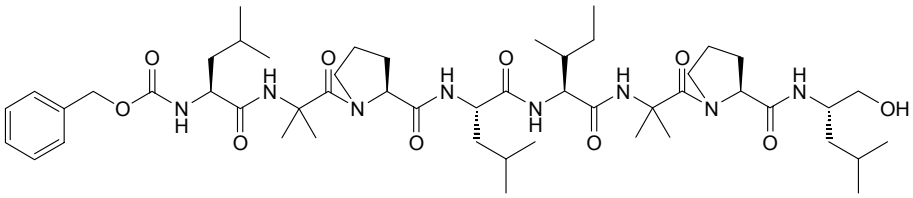
Synthesis of H-Leu-Ile-Aib-Pro-Leuol (73)



According to protocol D, to a solution of **78** (1033 mg, 1.565 mmol) in MeOH (60 ml), Pd/C was added and the mixture was stirred under an H_2 atmosphere overnight. After completion of the reaction and filtration through *Celite*, the solvent was evaporated to give **73** (821 mg, quant.). White foam. R_f(B) = 0.15. M.p. 76-77 °C. RP-HPLC (II): t_R 30.15, λ 220.

IR (KBr): 3311*vs,* 2959*vs,* 2874*s,* 1648*vs,* 1547*vs,* 1469*s,* 1408*s,* 1385*s,* 1366*s,* 1307*m,* 1243*w,* 1198*m,* 1172*m,* 1103*w,* 1068*w,* 1038*w,* 618*w.*
^{1}H-NMR (300 MHz, CD_3OD): 0.86-1.00 (*m*, 2 δCH_3^{Leu}, 2 δCH_3^{Leuol}, δCH_3^{Ile}, γCH_3^{Ile}), 1.15-1.33 (*m*, γCH_{2a}^{Ile}), 1.31-1.40 (*m*, βCH_{2a}^{Leu}, βCH_{2a}^{Leuol}), 1.46 (*br. s*, 2 βCH_3^{Aib}), 1.47-1.65 (*m*, βCH_{2b}^{Leu}, βCH_{2b}^{Leuol}, γCH_{2b}^{Ile}, γCH^{Leuol}), 1.66-1.78 (*m*, γCH^{Leu}), 1.79-2.00 (*m*, βCH_{2a}^{Pro}, γCH_2^{Pro}, βCH^{Ile}), 2.16-2.27 (*m*, βCH_{2b}^{Pro}), 3.50-3.64 (*m*, αCH^{Leu}, CH_2OH^{Leuol}), 3.64-3.74 (*m*, δCH_2^{Pro}), 3.97-4.07 (*m*, αCH^{Leuol}), 4.28 (*d*, *J* = 7.3, αCH^{Ile}), 4.43 (*dd*, *J* = 9.0, 5.3, αCH^{Pro}).
^{13}C-NMR (75.5 MHz, CD_3OD): 11.5 (*q*, δCH_3^{Ile}), 16.3 (*q*, γCH_3^{Ile}), 22.2 (*q*, δCH_3^{Leuol}), 22.2 (*q*, δCH_3^{Leu}), 23.5 (*q*, δCH_3^{Leuol}), 23.8 (*q*, δCH_3^{Leu}), 24.0 (*q*, βCH_3^{Aib}), 25.5 (*q*, βCH_3^{Aib}), 25.6 (*t*, γCH_2^{Pro}), 25.9 (*d*, γCH^{Leuol}), 26.1 (*d*, γCH^{Leuol}), 26.5 (*t*, γCH_2^{Ile}), 29.9 (*t*, βCH_2^{Pro}), 37.6 (*d*, βCH^{Ile}), 40.5 (*t*, βCH_2^{Leuol}), 45.2 (*t*, βCH_2^{Leu}), 49.5 (*t*, δCH_2^{Pro}), 50.9 (*d*, αCH^{Leuol}), 54.2 (*d*, αCH^{Ile}), 57.9 (*s*, αC^{Aib}), 58.9 (*d*, αCH^{Leu}), 64.1 (*d*, αCH^{Pro}), 65.8 (*t*, CH_2OH^{Leuol}), 173.3, 174.2, 174.4, 177.7 (4 *s*, 4 CO).
ESI-MS (MeOH+NaI): 548.3 (100%, $[M+Na]^+$).
Anal. calc. for $C_{27}H_{51}N_5O_5 \cdot 1.5\ H_2O$ (MG 552.73): C 58.67, H 9.85, N 12.67; found: C 58.84, H 9.73, N 12.48.
$[\alpha]_D^{20} = -46.7$ (*c* = 1.00, MeOH).

Synthesis of Z-Leu-Aib-Pro-Leu-Ile-Aib-Pro-Leuol (80)

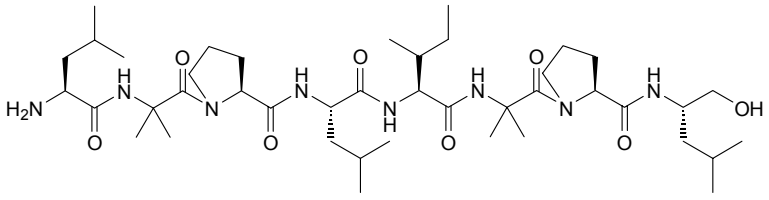


According to protocol A, a mixture of **73** (781.2 mg, 1.49 mmol), Z-Leu-Aib-Pro-OH **63** (665 mg, 1.49 mmol), TBTU (477.2 mg, 1.49 mmol), and HOBt (228.2 mg, 1.49 mmol) was dissolved in MeCN (110 ml), and TEA (627 µl, 4.46 mmol) was added. After completion of the reaction, the mixture was purified by flash chromatography to

give **80** (1381 mg, 97%). White foam. R_f(B) = 0.22, R_f(C) = 0.35. M.p. 116-117 °C. RP-HPLC (II): λ 218.

IR (KBr): 3442*vs,* 3296*vs,* 3036*m,* 2958*vs,* 2926*s,* 2874*s,* 1722*s,* 1648*vs,* 1539*vs,* 1469*s,* 1454*s,* 1415*s,* 1383*s,* 1365*s,* 1342*m,* 1304*m,* 1260*s,* 1220*m,* 1202*m,* 1172*s,* 1126*w,* 1047*m,* 746*w,* 719*w,* 700*m,* 682*w,* 657*w,* 643*w,* 620*m.*
^{1}H-NMR (600 MHz, CD_3OD): 0.82 (*t*, *J* = 7.2, δCH_3^{Ile}), 0.86-1.01 (*m*, 2 δCH_3^{Leu}, 2 δCH_3^{Leu}, 2 δCH_3^{Leuol}, γCH_3^{Ile}), 1.09-1.21 (*m*, γCH_{2a}^{Ile}), 1.24-1.40 (*m*, βCH_{2a}^{Leuol}), 1.42-1.46 (*m*, γCH_{2b}^{Ile}), 1.46, 1.47 (2 *br. s*, 4 βCH_3^{Aib}), 1.50-1.68 (*m*, βCH_2^{Leu}, βCH_{2b}^{Leu}, βCH_{2b}^{Leuol}, γCH^{Leu}, γCH^{Leu}, γCH^{Leuol}), 1.68-1.80 (*m*, βCH_{2b}^{Pro}, βCH_{2b}^{Pro}), 1.79-1.88 (*m*, γCH_{2a}^{Pro}, γCH_2^{Pro}), 1.88-2.00 (*m*, βCH^{Ile}, γCH_{2b}^{Pro}, βCH_{2a}^{Leu}), 2.20-2.33 (*m*, βCH_{2a}^{Pro}, βCH_{2a}^{Pro}), 3.23-3.31 (*m*, δCH_{2b}^{Pro}), 3.47-3.59 (*m*, δCH_{2ab}^{Pro}, CH_2OH^{Leuol}), 3.77-3.86 (*m*, δCH_{2a}^{Pro}), 3.92-4.05 (*m*, αCH^{Leuol}), 4.12 (*dd*, *J* = 8.7, 5.7, αCH^{Leu}), 4.16-4.25 (*m*, αCH^{Leu}), 4.25-4.36 (*m*, αCH^{Ile}, αCH^{Pro}), 4.43 (*t*, *J* = 7.7, αCH^{Pro}), 5.08, 5.19 (*AB*, *J* = 12.6, CH_2^Z), 7.27-7.43 (*m*, 5 arom. CH^Z).
^{13}C-NMR (150.9 MHz, CD_3OD): 11.7 (*q*, δCH_3^{Ile}), 16.3 (*q*, γCH_3^{Ile}), 21.0 (*q*, δCH_3^{Leu}), 21.2 (*q*, δCH_3^{Leu}), 22.1 (*q*, βCH_3^{Aib}), 23.4 (*q*, δCH_3^{Leu}), 23.5 (*q*, βCH_3^{Aib}), 23.7 (*q*, δCH_3^{Leu}), 24.1 (*q*, δCH_3^{Leuol}), 24.3 (*q*, δCH_3^{Leuol}), 25.8 (*q*, βCH_3^{Aib}), 26.0 (*d*, γCH^{Leuol}), 26.0 (*d*, γCH^{Leu}), 26.1 (*q*, βCH_3^{Aib}), 26.2 (*d*, γCH^{Leu}), 26.7 (*t*, γCH_2^{Ile}), 26.8 (*t*, γCH_2^{Pro}), 27.0 (*t*, γCH_2^{Pro}), 29.8 (*t*, βCH_2^{Pro}), 30.2 (*t*, βCH_2^{Pro}), 38.0 (*d*, βCH^{Ile}), 40.4 (*t*, βCH_2^{Leu}), 40.4 (*t*, βCH_2^{Leuol}), 41.3 (*t*, βCH_2^{Leu}), 49.9 (*t*, δCH_2^{Pro}), 50.3 (*t*, δCH_2^{Pro}), 51.2 (*d*, αCH^{Leuol}), 53.1 (*d*, αCH^{Leu}), 55.1 (*d*, αCH^{Leu}), 57.9 (*s*, 2 αC^{Aib}), 62.0 (*d*, αCH^{Ile}), 64.3 (*d*, αCH^{Pro}), 65.1 (*d*, αCH^{Pro}), 65.8 (*t*, CH_2OH^{Leuol}), 67.9 (*t*, CH_2^Z), 128.9, 129.2, 129.6 (3 *d*, 5 arom. CH^Z), 138.3 (*s*, arom. C^Z), 158.4 (*s*, $OCONH^Z$), 174.3, 174.4, 174.5, 174.7, 175.0, 175.8 (6 *s*, 7 CO).
ESI-MS (MeOH+NaI): 977.5 (100%, $[M+Na]^+$).
Anal. calc. for $C_{50}H_{82}N_8O_{10} \cdot H_2O$ (MG 973.22): C 61.71, H 8.70, N 11.51; found: C 61.72, H 8.73, N 11.48.
$[\alpha]_D^{20} = -5.5$ (*c* = 1.00, MeOH).

Synthesis of H-Leu-Aib-Pro-Leu-Ile-Aib-Pro-Leuol (81)

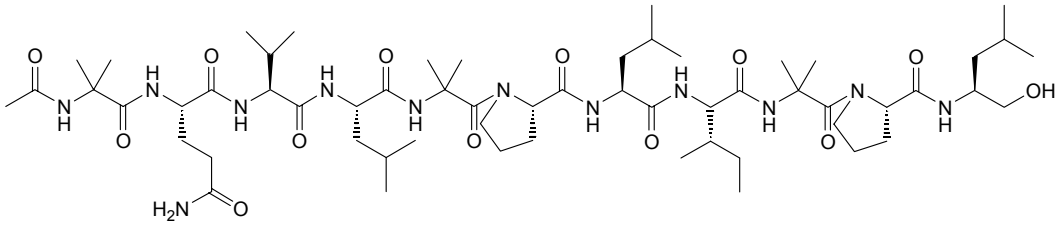


According to protocol D, to a solution of **80** (155 mg, 0.162 mmol) in MeOH (30 ml), Pd/C was added and the mixture was stirred under an H_2 atmosphere overnight. After completion of the reaction and filtration through *Celite*, the solvent was evaporated to give **81** (130.4 mg, 98%). White foam. R_f(C) = 0.22. M.p. 95-96 °C. RP-HPLC (II): t_R 85, λ 217.

IR (KBr): 3312*vs,* 2959*vs,* 2873*s,* 1647*vs,* 1536*vs,* 1469*vs,* 1412*vs,* 1384*s,* 1365*s,* 1340*m,* 1286*s,* 1245*m,* 1196*s,* 1172*s,* 1147*m,* 1101*w,* 1069*m,* 1039*w,* 673*w,* 618*m.*

^{1}H-NMR (300 MHz, CD_3OD): 0.84 (*t*, *J* = 7.4, δCH_3^{Ile}), 0.87-1.03 (*m*, 2 δCH_3^{Leu}, 2 δCH_3^{Leu}, 2 δCH_3^{Leuol}, γCH_3^{Ile}), 1.28-1.44 (*m*, βCH_{2b}^{Leu}, γCH_{2b}^{Ile}), 1.44-1.69 (*m*, γCH_{2a}^{Ile}, βCH_{2b}^{Leu}, βCH_2^{Leuol}, βCH_{2a}^{Leu}, γCH^{Leu}), 1.47, 1.48, 1.50, 1.53 (4 *br. s*, 4 βCH_3^{Aib}), 1.69-1.85 (*m*, βCH_{2b}^{Pro}, βCH_{2b}^{Pro}, γCH^{Leu}, γCH^{Leuol}), 1.85-2.04 (*m*, βCH_{2a}^{Leu}, γCH_2^{Pro}, βCH^{Ile}, γCH_2^{Pro}), 2.21-2.43 (*m*, βCH_{2a}^{Pro}, βCH_{2a}^{Pro}), 3.24-3.32 (*m*, δCH_{2b}^{Pro}), 3.39-3.45 (*m*, αCH^{Leu}), 3.46-3.60 (*m*, δCH_{2b}^{Pro}, CH_2OH^{Leuol}), 3.78-4.02 (*m*, δCH_{2a}^{Pro}, δCH_{2a}^{Pro}, αCH^{Leuol}), 4.21 (*dd*, *J* = 11.3, 3.8, αCH^{Leu}), 4.28-4.40 (*m*, αCH^{Ile}, αCH^{Pro}), 4.44 (*t*, *J* = 7.5, αCH^{Pro}).
^{13}C-NMR (75.5 MHz, CD_3OD): 10.8 (*q*, δCH_3^{Ile}), 16.1 (*q*, γCH_3^{Ile}), 21.2 (*q*, δCH_3^{Leu}), 22.1 (*q*, δCH_3^{Leu}), 22.2 (*q*, βCH_3^{Aib}), 23.6 (*q*, δCH_3^{Leu}), 23.8 (*q*, βCH_3^{Aib}), 24.0 (*q*, δCH_3^{Leu}), 24.0 (*q*, δCH_3^{Leuol}), 24.3 (*q*, δCH_3^{Leuol}), 25.8 (*q*, βCH_3^{Aib}), 26.0 (*d*, γCH^{Leuol}), 26.3 (*d*, γCH^{Leu}), 26.4 (*q*, βCH_3^{Aib}), 26.4 (*d*, γCH^{Leu}), 26.7 (*t*, γCH_2^{Ile}), 26.9 (*t*, γCH_2^{Pro}), 27.1 (*t*, γCH_2^{Pro}), 30.1 (*t*, βCH_2^{Pro}), 30.2 (*t*, βCH_2^{Pro}), 37.8 (*d*, βCH^{Ile}), 40.5 (*t*, βCH_2^{Leuol}), 40.7 (*t*, βCH_2^{Leu}), 45.4 (*t*, βCH_2^{Leu}), 50.1 (*t*, δCH_2^{Pro}), 50.3 (*t*, δCH_2^{Pro}), 51.2 (*d*, αCH^{Leuol}), 54.6 (*d*, αCH^{Leu}), 55.0 (*d*, αCH^{Leu}), 57.4, 58.1 (2 *s*, 2 αC^{Aib}), 59.3 (*d*, αCH^{Ile}), 64.5 (*d*, αCH^{Pro}), 65.0 (*d*, αCH^{Pro}), 65.8 (*t*, CH_2OH^{Leuol}), 173.2, 174.4, 174.6, 175.1, 175.4, 175.8, 178.6 (7 *s*, 7 CO).
ESI-MS (MeOH+NaI): 843.5 (100%, $[M+Na]^+$).
Anal. calc. for $C_{42}H_{76}N_8O_8 \cdot 0.5\ H_2O$ (MG 831.11): C 60.77, H 9.35, N 13.50; found: C 60.75, H 9.35, N 13.50.
$[\alpha]_D^{20} = -1.4$ (*c* = 1.00, MeOH).

Synthesis of *Hypomurocin A3*, Ac-Aib-Gln-Val-Leu-Aib-Pro-Leu-Ile-Aib-Pro-Leuol (6)



According to protocol A, a mixture of **81** (130.4 mg, 0.16 mmol), Ac-Aib-Gln-Val-OH **51** (59.1 mg, 0.16 mmol), TBTU (51 mg, 0.16 mmol), and HOBt (24.4 mg, 0.16 mmol) was dissolved in MeCN (15 ml), and TEA (67 µl, 0.48 mmol) was added. After completion of the reaction, the mixture was purified by flash chromatography to give **6** (126 mg, 68%). White foam. R_f(C) = 0.26. M.p. 239-240 °C. RP-HPLC (II): t_R 117, λ 217.

IR (KBr): 3394*vs,* 3059*w,* 2959*vs,* 2928*vs,* 2874*s,* 1647*vs,* 1539*vs,* 1469*vs,* 1440*s,* 1415*s,* 1384*s,* 1364*s,* 1290*s,* 1265*s,* 1219*s,* 1193*s,* 1172*s,* 1126*m,* 1094*m,* 1050*m,* 1030*m,* 688*w,* 618*s.*
^{1}H-NMR (600 MHz, CD_3OD): 0.84 (*t*, *J* = 7.3, δCH_3^{Ile}), 0.87 (*d*, *J* = 6.4, δCH_3^{Leu}), 0.90-0.94 (*m*, δCH_3^{Leu}, δCH_3^{Leuol}, δCH_3^{Leu}), 0.95-0.98 (*m*, γCH_3^{Ile}, δCH_3^{Leuol}), 1.00-1.03 (*m*, γCH_3^{Val}, δCH_3^{Leu}), 1.06 (*d*, *J* = 6.9, γCH_3^{Val}), 1.28-1.38 (*m*, γCH_{2b}^{Ile}, βCH_{2b}^{Leuol}), 1.47, 1.48, 1.49 (3 *br. s*, 6 βCH_3^{Aib}), 1.49-1.55 (*m*, γCH_{2a}^{Ile}), 1.57-1.64 (*m*, βCH_{2a}^{Leuol},

βCH$_{2b}^{Leu}$, βCH$_{2b}^{Leu}$), 1.64-1.69 (*m*, γCH^{Leuol}), 1.71-1.81 (*m*, γCH^{Leu}, βCH$_{2b}^{Pro}$, βCH$_{2b}^{Pro}$, βCH$_{2a}^{Leu}$), 1.81-1.86 (*m*, γCH^{Leu}), 1.86-1.91 (*m*, γCH$_{2b}^{Pro}$), 1.91-2.02 (*m*, βCH$_{2a}^{Leu}$, βCH^{Ile}, γCH$_{2a}^{Pro}$, γCH$_{2b}^{Pro}$), 2.05-2.08 (*m*, γCH$_{2a}^{Pro}$), 2.08-2.15 (*m*, βCH$_2^{Gln}$), 2.24-2.31 (*m*, βCH$_{2a}^{Pro}$, βCH^{Val}), 2.31-2.38 (*m*, βCH$_{2a}^{Pro}$), 2.38-2.45 (*m*, γCH$_{2b}^{Gln}$), 2.45-2.51 (*m*, γCH$_{2a}^{Gln}$), 3.25-3.30 (*m*, δCH$_{2b}^{Pro}$), 3.50, 3.56 (*ABX*, *J* = 11.3, *J* = 5.7, C*H*$_2$OH^{Leuol}), 3.58-3.63 (*m*, δCH$_{2b}^{Pro}$), 3.80-3.86 (*m*, δCH$_{2a}^{Pro}$, δCH$_{2a}^{Pro}$), 3.94 (*d*, *J* = 8.3, αCH^{Val}), 3.94-3.99 (*m*, αCH^{Leuol}), 4.06 (*dd*, *J* = 7.7, 4.7, αCH^{Gln}), 4.20 (*dd*, *J* = 11.7, 3.7, αCH^{Leu}), 4.28 (*d*, *J* = 7.9, αCH^{Ile}), 4.33-4.37 (*m*, αCH^{Pro}, αCH^{Leu}), 4.44 (*t*, *J* = 7.7, αCH^{Pro}).

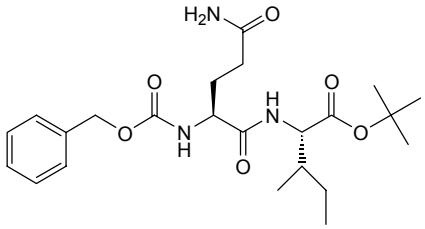
^{13}C-NMR (150.9 MHz, CD_3OD): 10.9 (*q*, δCH$_3^{Ile}$), 16.0 (*q*, γCH$_3^{Ile}$), 19.7, 20.0 (2 *q*, 2 γCH$_3^{Val}$), 21.1 (*q*, δCH$_3^{Leu}$), 21.2 (*q*, δCH$_3^{Leuol}$), 22.1 (*q*, δCH$_3^{Leu}$), 23.2 (*q*, CH$_3^{Ac}$), 23.5 (*q*, δCH$_3^{Leu}$), 23.9 (*q*, δCH$_3^{Leu}$), 24.1 (*q*, δCH$_3^{Leuol}$), 24.1 (*q*, βCH$_3^{Aib}$), 24.2 (*q*, βCH$_3^{Aib}$), 25.7 (*d*, γCH^{Leu}), 26.0 (*d*, γCH^{Leuol}), 26.2 (*d*, γCH^{Leu}), 26.3 (*t*, γCH$_2^{Ile}$), 26.4 (*t*, βCH$_2^{Gln}$), 26.4 (*q*, 2 βCH$_3^{Aib}$), 26.7 (*t*, γCH$_2^{Pro}$), 26.7 (*q*, βCH$_3^{Aib}$), 26.9 (*t*, γCH$_2^{Pro}$), 27.0 (*q*, βCH$_3^{Aib}$), 30.2 (*t*, βCH$_2^{Pro}$), 30.2 (*t*, βCH$_2^{Pro}$), 30.5 (*d*, βCH^{Val}), 32.2 (*t*, γCH$_2^{Gln}$), 37.8 (*d*, βCH^{Ile}), 40.4 (*t*, βCH$_2^{Leuol}$), 40.7 (*t*, βCH$_2^{Leu}$), 41.2 (*t*, βCH$_2^{Leu}$), 50.3 (*t*, δCH$_2^{Pro}$), 50.6 (*t*, δCH$_2^{Pro}$), 51.1 (*d*, αCH^{Leuol}), 53.9 (*d*, αCH^{Leu}), 55.0 (*d*, αCH^{Leu}), 57.4 (*s*, αC^{Aib}), 57.8 (*s*, αC^{Aib}), 57.8 (*d*, αCH^{Gln}), 58.0 (*s*, αC^{Aib}), 59.4 (*d*, αCH^{Ile}), 63.0 (*d*, αCH^{Val}), 64.4 (*d*, αCH^{Pro}), 64.9 (*d*, αCH^{Pro}), 65.8 (*t*, CH$_2$OH^{Leuol}), 173.2, 173.7, 174.3, 174.6, 175.0, 175.5, 175.9, 175.8, 178.0, 178.5 (10 *s*, 12 CO).

ESI-MS (MeOH+NaI): 1197.6 (100%, [*M*+Na]$^+$).

Anal. calc. for $C_{58}H_{102}N_{12}O_{13} \cdot H_2O$ (MG 1193.52): C 58.37, H 8.78, N 14.08; found: C 58.26, H 9.00, N 13.93.

$[\alpha]_D^{20} = -9.0$ (*c* = 1.00, MeOH).

Synthesis of Z-Gln-Ile-O*t*Bu (54)



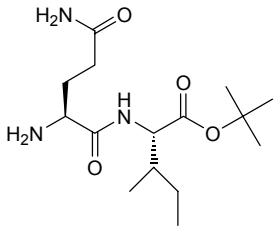
According to protocol A, a mixture of Z-Gln-OH (62.6 mg, 0.223 mmol), H-Ile-O*t*Bu · HCl (50 mg, 0.223 mmol), TBTU (71.7 mg, 0.223 mmol), and HOBt (34.3 mg, 0.223 mmol) was dissolved in MeCN (14 ml), and TEA (94 µl, 0.67 mmol) was added. After completion of the reaction, the mixture was purified by flash chromatography to give **54** (91.9 mg, 92%). White powder. R_f(B) = 0.80. M.p. 146 °C. RP-HPLC (II): t_R 23.05, λ 213.

IR (KBr): 3411*s,* 3316*s,* 2969*m,* 2920*m,* 2855*w,* 1724*s,* 1689*s,* 1651*vs,* 1543*s,* 1455*m,* 1400*w,* 1369*m,* 1329*w,* 1289*m,* 1263*m,* 1168*m,* 1139*m,* 1088*w,* 1061*m,* 976*w,* 743*w,* 695*w,* 665*w,* 624*w.*

^{1}H-NMR (300 MHz, CD_3OD): 0.89-0.96 (*m*, γCH$_3^{Ile}$, δCH$_3^{Ile}$), 1.19-1.32 (*m*, γCH$_{2a}^{Ile}$), 1.42-1.54 (*m*, γCH$_{2b}^{Ile}$), 1.46 (*s*, 3 CH$_3^{OtBu}$), 1.81-1.95 (*m*, βCH$_{2b}^{Gln}$, βCH^{Ile}), 2.01-2.14

(*m*, βCH_{2a}^{Gln}), 2.34 (*t*, *J* = 7.6, γCH_2^{Gln}), 4.18-4.27 (*m*, αCH^{Gln}, αCH^{Ile}), 5.09 (*br*. *s*, CH_2^Z), 7.25-7.38 (*m*, 5 arom. CH^Z).
^{13}C-NMR (75.5 MHz, CD_3OD): 11.9 (*q*, δCH_3^{Ile}), 16.0 (*q*, γCH_3^{Ile}), 26.4 (*t*, γCH_2^{Ile}), 28.4 (*q*, 3 CH_3^{OtBu}), 29.3 (*t*, γCH_2^{Gln}), 32.6 (*t*, βCH_2^{Gln}), 38.5 (*d*, βCH^{Ile}), 55.7 (*d*, αCH^{Gln}), 59.0 (*d*, αCH^{Ile}), 67.8 (*t*, CH_2^Z), 83.0 (*s*, C^{OtBu}), 128.9, 129.0, 129.5 (3 *d*, 5 arom. CH^Z), 172.1, 177.9 (2 *s*, 3 CO). The $OCONH^Z$ and arom. C^Z could not be identified.
ESI-MS (MeOH+NaI): 921.4 (15%, $[2M+Na]^+$), 488.2 (20%, $[M+K]^+$), 472.2 (100%, $[M+Na]^+$).
Anal. calc. for $C_{23}H_{35}N_3O_6$ (MG 449.54): C 61.45, H 7.85, N 9.35; found: C 61.35, H 7.90, N 9.34.
$[\alpha]_D^{20} = -19.1$ (*c* = 1.56, MeOH).

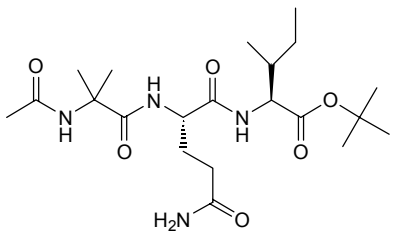
Synthesis of H-Gln-Ile-O*t*Bu (56)



According to protocol D, to a solution of **54** (638.7 mg, 1.421 mmol) in MeOH (42 ml), Pd/C was added and the mixture was stirred under an H_2 atmosphere overnight. After completion of the reaction and filtration through *Celite*, the solvent was evaporated to give **56** (448 mg, quant.). White foam. R_f(B) = 0.09. RP-HPLC (II): t_R 19.27, λ 203.

^{1}H-NMR (300 MHz, CD_3OD): 0.92-0.99 (*m*, γCH_3^{Ile}, δCH_3^{Ile}), 1.20-1.36 (*m*, γCH_{2a}^{Ile}), 1.42-1.56 (*m*, γCH_{2b}^{Ile}), 1.47 (*s*, 3 CH_3^{OtBu}), 1.84-1.98 (*m*, βCH_{2b}^{Gln}, βCH^{Ile}), 1.98-2.11 (*m*, βCH_{2a}^{Gln}), 2.42 (*t*, *J* = 7.8, γCH_2^{Gln}), 3.68 (*t*, *J* = 6.4, αCH^{Gln}), 4.27 (*d*, *J* = 5.4, αCH^{Ile}).
^{13}C-NMR (75.5 MHz, CD_3OD): 11.9 (*q*, δCH_3^{Ile}), 16.0 (*q*, γCH_3^{Ile}), 26.4 (*t*, γCH_2^{Ile}), 28.3 (3 *q*, CH_3^{OtBu}), 31.0 (*t*, γCH_2^{Gln}), 32.3 (*t*, βCH_2^{Gln}), 38.4 (*d*, βCH^{Ile}), 54.7 (*d*, αCH^{Gln}), 58.9 (*d*, αCH^{Ile}), 83.1 (*s*, C^{OtBu}), 172.1, 174.5, 178.0 (3 *s*, 3 CO).

Synthesis of Ac-Aib-Gln-Ile-O*t*Bu (58)

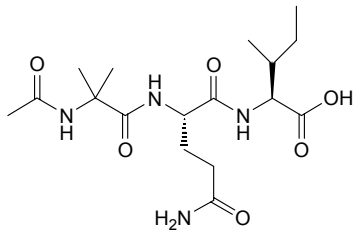


According to protocol A, a mixture of **56** (338 mg, 1.072 mmol), Ac-Aib-OH (155.6 mg, 1.072 mmol), TBTU (344.2 mg, 1.072 mmol), and HOBt (164.6 mg, 1.072 mmol)

was dissolved in MeCN (65 ml), and TEA (452 µl, 3.215 mmol) was added. After completion of the reaction, the mixture was purified by flash chromatography to give **58** (311.2 mg, 66%). White foam. R_f(B) = 0.30. M.p. 80-85 °C. RP-HPLC (II): t_R 18.09, λ 203.

IR (KBr): 3417*vs,* 3064*w,* 2973*s,* 2935*s,* 2878*w,* 1731*vs,* 1660*vs,* 1536*vs,* 1454*s,* 1383*s,* 1368*s,* 1300*s,* 1257*m,* 1221*s,* 1147*vs,* 1038*w,* 987*w,* 847*w,* 589*m.*
^{1}H-NMR (300 MHz, CD_3OD): 0.89-0.97 (*m*, γCH_3^{Ile}, δCH_3^{Ile}), 1.16-1.32 (*m*, γCH_{2a}^{Ile}), 1.43-1.57 (*m*, γCH_{2b}^{Ile}), 1.43, 1.45 (2 *s*, 2 CH_3^{Aib}), 1.46 (*s*, 3 CH_3^{OtBu}), 1.85-2.02 (*m*, βCH_{2b}^{Gln}, βCH^{Ile}), 1.97 (*s*, CH_3^{Ac}), 2.08-2.20 (*m*, βCH_{2a}^{Gln}), 2.31 (*t*, *J* = 7.1, γCH_2^{Gln}), 4.16 (*d*, *J* = 6.3, αCH^{Ile}), 4.36 (*dd*, *J* = 9.6, 4.5, αCH^{Gln}).
^{13}C-NMR (75.5 MHz, CD_3OD): 11.8 (*q*, δCH_3^{Ile}), 15.9 (*q*, γCH_3^{Ile}), 23.2 (*q*, βCH_3^{Aib}), 25.6 (*q*, βCH_3^{Aib}), 25.7 (*q*, CH_3^{Ac}), 26.6 (*t*, γCH_2^{Ile}), 28.4 (*q*, 3 CH_3^{OtBu}), 32.5 (*t*, βCH_2^{Gln}, γCH_2^{Gln}), 38.0 (*d*, βCH^{Ile}), 54.4 (*d*, αCH^{Gln}), 57.7 (*s*, αC^{Aib}), 59.2 (*d*, αCH^{Ile}), 82.8 (*s*, C^{OtBu}), 172.0, 173.2, 173.9, 177.0, 178.1 (5 *s*, 5 CO).
ESI-MS (MeOH+NaI): 465.2 (100%, $[M+Na]^+$).
Anal. calc. for $C_{21}H_{38}N_4O_6 \cdot 0.5\ H_2O$ (MG 451.56): C 55.86, H 8.71, N 12.41; found: C 55.94, H 8.54, N 12.41.
$[\alpha]_D^{20} = -26.4$ (*c* = 2.97, MeOH).

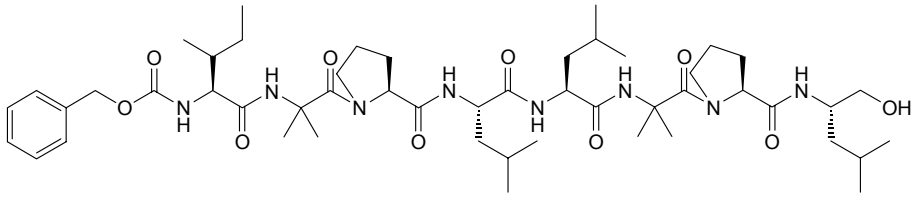
Synthesis of Ac-Aib-Gln-Ile-OH (52)



At 0 °C, **58** (275 mg, 0.621 mmol) was dissolved in TFA (15 ml) under an N_2 atmosphere. After 2.5 h, the reaction was quenched by evaporating the TFA. The residue was dissolved in a mixture of CH_2Cl_2/Et_2O 1:1 (v/v) and evaporated (3x) to give **52** (238 mg, quant.). White powder. R_f(B) = 0.15, R_f(C) = 0.26. RP-HPLC (II): t_R 30.46, λ 205.

IR (KBr): 3437*vs,* 2926*s,* 2853*m,* 1659*vs,* 1541*vs,* 1455*s,* 1204*vs,* 1139*s,* 835*m,* 802*m,* 723*m,* 673*m,* 619*m.*
^{1}H-NMR (300 MHz, CD_3OD): 0.89-0.98 (*m*, γCH_3^{Ile}, δCH_3^{Ile}), 1.18-1.33 (*m*, γCH_{2a}^{Ile}), 1.45-1.58 (*m*, γCH_{2b}^{Ile}), 1.44, 1.45 (2 *s*, 2 CH_3^{Aib}), 1.88-2.01 (*m*, βCH_{2b}^{Gln}, βCH^{Ile}), 1.96 (*s*, CH_3^{Ac}), 2.07-2.19 (*m*, βCH_{2a}^{Gln}), 2.31 (*t*, *J* = 7.1, γCH_2^{Gln}), 4.31 (*d*, *J* = 6.0, αCH^{Ile}), 4.37 (*dd*, *J* = 9.2, 4.4, αCH^{Gln}).
^{13}C-NMR (75.5 MHz, CD_3OD): 11.8 (*q*, δCH_3^{Ile}), 16.0 (*q*, γCH_3^{Ile}), 23.1 (*q*, βCH_3^{Aib}), 25.3 (*q*, βCH_3^{Aib}), 25.9 (*q*, CH_3^{Ac}), 26.4 (*t*, γCH_2^{Ile}), 28.4 (*t*, βCH_2^{Gln}), 32.5 (*t*, γCH_2^{Gln}), 38.1 (*d*, βCH^{Ile}), 54.4 (*d*, αCH^{Gln}), 57.8 (*s*, αC^{Aib}), 58.5 (*d*, αCH^{Ile}), 82.8 (*s*, C^{OtBu}), 173.2, 173.9, 174.4, 177.1, 178.2 (5 *s*, 5 CO).
ESI-MS (MeOH+NaI): 409.3 (100%, $[M+Na]^+$), 387.3 (20%, $[M+H]^+$).

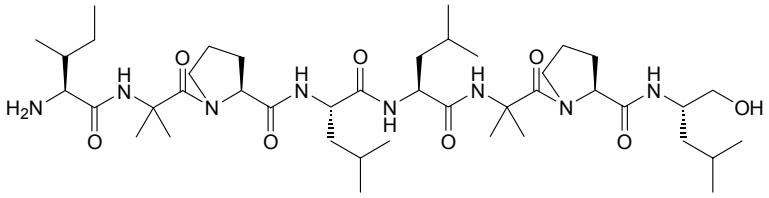
Synthesis of Z-Ile-Aib-Pro-Leu-Leu-Aib-Pro-Leuol (82)



According to protocol A, a mixture of Z-Ile-Aib-Pro-OH (**75**) (546 mg, 1.22 mmol), H-Leu-Leu-Aib-Pro-Leuol (**67**) (641 mg, 1.22 mmol), TBTU (392 mg, 1.22 mmol), and HOBt (187.3 mg, 1.22 mmol) was dissolved in MeCN (70 ml), and TEA (514 µl, 3.66 mmol) was added. After completion of the reaction, the mixture was purified by flash chromatography to give **82** (739 mg, 63%). White foam. R_f(B) = 0.50. M.p. 144 °C. RP-HPLC (II): t_R 58.21, λ 205.

IR (KBr): 3441*vs,* 2960*s,* 2872*m,* 1726*s,* 1624*vs,* 1529*vs,* 1468*s,* 1412*s,* 1385*s,* 1365*m,* 1339*m,* 1253*s,* 1218*s,* 1171*s,* 1126*m,* 1100*m,* 1039*m,* 750*w,* 699*w,* 616*m.*
^{1}H-NMR (300 MHz, CD_3OD): 0.82-0.99 (*m*, γCH_3^{Ile}, δCH_3^{Ile}, 2 δCH_3^{Leuol}, 2 δCH_3^{Leu}, 2 δCH_3^{Leu}), 1.21-1.31 (*m*, γCH_{2a}^{Ile}), 1.32-1.40 (*m*, βCH_{2a}^{Leuol}), 1.45, 1.48 (2 *br. s*, 4 βCH_3^{Aib}), 1.51-1.57 (*m*, γCH_{2b}^{Ile}), 1.57-1.61 (*m*, βCH_{2b}^{Leu}, βCH_{2b}^{Leuol}), 1.62-1.71 (*m*, βCH_2^{Leu}, γCH^{Leu}, γCH^{Leuol}), 1.71-1.79 (*m*, βCH_{2b}^{Pro}, βCH_{2b}^{Pro}, γCH^{Leu}), 1.79-1.88 (*m*, γCH_2^{Pro}, γCH_{2b}^{Pro}), 1.88-2.00 (*m*, βCH_{2a}^{Leu}, βCH^{Ile}, γCH_{2a}^{Pro}), 2.21-2.32 (*m*, βCH_{2a}^{Pro}, βCH_{2a}^{Pro}), 3.28-3.37 (*m*, δCH_{2b}^{Pro}), 3.46-3.64 (*m*, CH_2OH^{Leuol}, δCH_{2ab}^{Pro}), 3.79-3.86 (*m*, δCH_{2a}^{Pro}), 3.91-4.03 (*m*, αCH^{Ile}, αCH^{Leuol}), 4.18 (*dd*, *J* = 11.5, 4.0, αCH^{Leu}), 4.28-4.35 (*m*, αCH^{Pro}), 4.40-4.49 (*m*, αCH^{Pro}, αCH^{Leu}), 5.09, 5.20 (*AB*, *J* = 12.6, CH_2^Z), 7.28-7.50 (*m*, 5 arom. CH^Z).
^{13}C-NMR (75.5 MHz, CD_3OD): 11.7 (*q*, δCH_3^{Ile}), 16.3 (*q*, γCH_3^{Ile}), 21.0 (*q*, δCH_3^{Leu}), 21.2 (*q*, δCH_3^{Leu}), 22.1, 23.5 (2 *q*, 2 δCH_3^{Leuol}), 23.6 (*q*, δCH_3^{Leu}), 23.7 (*q*, δCH_3^{Leu}), 24.1, 24.3 (2 *q*, 2 βCH_3^{Aib}), 25.8 (*d*, γCH^{Leu}), 26.0 (*d*, γCH^{Leuol}), 26.1 (*q*, βCH_3^{Aib}), 26.2 (*d*, γCH^{Leu}), 26.7 (*t*, γCH_2^{Ile}, γCH_2^{Pro}), 26.8 (*q*, βCH_3^{Aib}), 27.0 (*t*, γCH_2^{Pro}), 29.9 (*t*, βCH_2^{Pro}), 30.2 (*t*, βCH_2^{Pro}), 38.0 (*d*, βCH^{Ile}), 40.4 (*t*, βCH_2^{Leu}, βCH_2^{Leuol}), 41.3 (*t*, βCH_2^{Leu}), 49.5 (*t*, δCH_2^{Pro}), 50.3 (*t*, δCH_2^{Pro}), 51.3 (*d*, αCH^{Leuol}), 53.2 (*d*, αCH^{Leu}), 55.1 (*d*, αCH^{Leu}), 57.9 (*s*, 2 αC^{Aib}), 62.1 (*d*, αCH^{Ile}), 64.4 (*d*, αCH^{Pro}), 65.1 (*d*, αCH^{Pro}), 65.8 (*t*, CH_2OH^{Leuol}), 67.9 (*t*, CH_2^Z), 129.0, 129.2, 129.6 (3 *d*, 5 arom. CH^Z), 138.3 (*s*, arom. C^Z), 158.4 (*s*, $OCONH^Z$), 174.3, 174.4, 174.5, 174.7, 175.0, 175.9, 176.0 (7 *s*, 7 CO).
ESI-MS (MeOH+NaI): 977.6 (100%, $[M+Na]^+$), 500.3 (100%, $[M+2Na]^{2+}$).
Anal. calc. for $C_{50}H_{82}N_8O_{10} \cdot 2\ H_2O$ (MG 991.27): C 60.58, H 8.75, N 11.30; found: C 60.83, H 8.42, N 11.28.
$[\alpha]_D^{20} = -4.5$ (*c* = 2.40, MeOH).

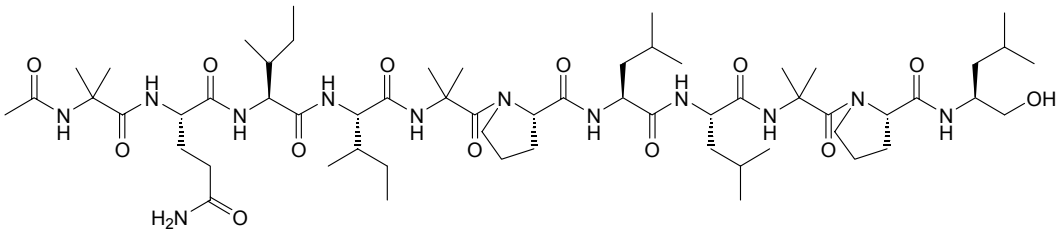
Synthesis of H-Ile-Aib-Pro-Leu-Leu-Aib-Pro-Leuol (83)



According to protocol D, to a solution of **82** (14 mg, 0.015 mmol) in MeOH (10 ml), Pd/C was added and the mixture was stirred under an H_2 atmosphere overnight. After completion of the reaction and filtration through *Celite*, the solvent was evaporated to give **83** (12 mg, quant.). White foam. R_f(B) = 0.08. M.p. 100-105 °C. RP-HPLC (II): t_R 92.86, λ 207.

IR (KBr): 3311*vs,* 2959*vs,* 2873*s,* 1644*vs,* 1536*vs,* 1470*s,* 1412*vs,* 1385*s,* 1365*s,* 1340*m,* 1283*m,* 1196*m,* 1172*s,* 1101*w,* 1069*w,* 1038*w,* 618*m.*
^{1}H-NMR (300 MHz, CD_3OD): 0.82-1.02 (*m*, γCH_3^{Ile}, δCH_3^{Ile}, 2 δCH_3^{Leuol}, 2 δCH_3^{Leu}, 2 δCH_3^{Leu}), 1.11-1.42 (*m*, γCH_{2a}^{Ile}, βCH_{2a}^{Leuol}), 1.44, 1.48, 1.51, 1.54 (4 *br. s*, 4 βCH_3^{Aib}), 1.54-1.83 (*m*, γCH_{2b}^{Ile}, βCH_{2b}^{Leuol}, βCH_{2b}^{Leu}, γCH^{Leu}, βCH_{2b}^{Pro}, βCH_{2b}^{Pro}, βCH^{Ile}, γCH^{Leuol}, βCH_{2b}^{Leu}, γCH^{Leu}), 1.83-2.05 (*m*, βCH_{2a}^{Leu}, γCH_2^{Pro}, γCH_2^{Pro}, βCH_{2a}^{Leu}), 2.20-2.32 (*m*, βCH_{2a}^{Pro}), 2.32-2.42 (*m*, βCH_{2a}^{Pro}), 3.29-3.38 (*m*, δCH_{2b}^{Pro}, αCH^{Ile}), 3.43-3.59 (*m*, CH_2OH^{Leuol}, δCH_{2b}^{Pro}), 3.77-4.02 (*m*, δCH_{2a}^{Pro}, δCH_{2a}^{Pro}, αCH^{Leuol}), 4.18 (*dd*, *J* = 11.4, 4.2, αCH^{Leu}), 4.31 (*t*, *J* = 8.0, αCH^{Pro}), 4.40-4.48 (*m*, αCH^{Pro}, αCH^{Leu}).
^{13}C-NMR (75.5 MHz, CD_3OD): 12.4 (*q*, δCH_3^{Ile}), 16.4 (*q*, γCH_3^{Ile}), 21.1 (*q*, δCH_3^{Leu}), 21.3 (*q*, δCH_3^{Leu}), 22.2, 23.5 (2 *q*, 2 δCH_3^{Leuol}), 23.6 (*q*, δCH_3^{Leu}), 23.7 (*q*, δCH_3^{Leu}), 24.2, 24.4 (2 *q*, 2 βCH_3^{Aib}), 25.2 (*d*, γCH^{Leu}), 25.8 (*d*, γCH^{Leuol}), 26.0 (*q*, βCH_3^{Aib}), 26.2 (*d*, γCH^{Leu}), 26.2 (*t*, γCH_2^{Ile}), 26.7 (*t*, γCH_2^{Pro}), 26.8 (*q*, βCH_3^{Aib}), 26.8 (*t*, γCH_2^{Pro}), 30.0 (*t*, βCH_2^{Pro}), 30.1 (*t*, βCH_2^{Pro}), 39.9 (*d*, βCH^{Ile}), 40.3 (*t*, βCH_2^{Leu}, βCH_2^{Leuol}), 41.2 (*t*, βCH_2^{Leu}), 49.9 (*t*, δCH_2^{Pro}), 50.2 (*t*, δCH_2^{Pro}), 51.2 (*d*, αCH^{Leuol}), 53.1 (*d*, αCH^{Leu}), 55.0 (*d*, αCH^{Leu}), 57.5, 57.8 (2 *s*, 2 αC^{Aib}), 60.8 (*d*, αCH^{Ile}), 64.3 (*d*, αCH^{Pro}), 65.1 (*d*, αCH^{Pro}), 65.8 (*t*, CH_2OH^{Leuol}), 174.2, 174.4, 174.6, 175.3, 175.8, 177.5 (6 *s*, 7 CO).
ESI-MS (MeOH+NaI): 843.6 (100%, $[M+Na]^+$).
Anal. calc. for $C_{42}H_{76}N_8O_8 \cdot H_2O$ (MG 839.12): C 60.12, H 9.37, N 13.35; found: C 59.96, H 9.13, N 13.15.
$[\alpha]_D^{20} = -5.4$ (*c* = 1.20, MeOH).

Synthesis of *Hypomurocin A5*, Ac-Aib-Gln-Ile-Ile-Aib-Pro-Leu-Leu-Aib-Pro-Leuol (7)



According to protocol A, a mixture of **85** (440 mg, 0.536 mmol), **52** (207 mg, 1.22 mmol), TBTU (172 mg, 0.536 mmol), and HOBt (187.3 mg, 0.536 mmol) was dissolved in MeCN (30 ml), and TEA (226 µl, 1.608 mmol) was added. After completion of the reaction, the mixture was purified by flash chromatography to give **7** (195.3 mg, 31%). White foam. R_f(C) = 0.28. M.p. 156-158 °C. RP-HPLC (II): t_R 49.51, λ 203.

IR (KBr): 3436*vs,* 2960*s,* 2875*m,* 1641*vs,* 1539*s,* 1469*m,* 1415*m,* 1385*m,* 1365*m,* 1341*m,* 1292*m,* 1171*m,* 1101*m,* 1068*m,* 614*m.*
^{1}H-NMR (300 MHz, CD_3OD): 0.83-0.99 (*m*, δCH_3^{Leu}, δCH_3^{Ile}, δCH_3^{Leu}, δCH_3^{Leuol}, δCH_3^{Leu}, δCH_3^{Ile}, δCH_3^{Leuol}, γCH_3^{Ile}, γCH_3^{Ile}), 1.01 (*d*, *J* = 7.0, δCH_3^{Leu}), 1.27-1.39 (*m*, βCH_{2b}^{Leuol}, γCH_{2b}^{Ile}, γCH_{2b}^{Ile}), 1.46, 1.49, 1.51, 1.55 (4 *br. s*, 8 CH_3^{Aib}), 1.54-1.68 (*m*, γCH^{Leuol}, βCH_{2a}^{Leuol}, γCH_{2a}^{Ile}, γCH_{2a}^{Ile}, βCH_{2b}^{Leu}, βCH_{2b}^{Leu}), 1.68-1.82 (*m*, βCH_{2a}^{Leu}, βCH_{2b}^{Pro}, γCH^{Leu}, βCH_{2b}^{Pro}), 1.83-1.91 (*m*, γCH_{2b}^{Pro}, γCH^{Leu}), 1.93-2.01 (*m*, γCH_{2b}^{Pro}, γCH_{2a}^{Pro}, βCH^{Ile}), 2.01-2.15 (*m*, βCH_2^{Gln}, γCH_{2a}^{Pro}, βCH^{Ile}, βCH_{2a}^{Leu}), 2.25-2.31 (*m*, βCH_{2a}^{Pro}), 2.31-2.37 (*m*, βCH_{2a}^{Pro}), 2.38-2.43 (*m*, γCH_{2b}^{Gln}), 2.45-2.51 (*m*, γCH_{2a}^{Gln}), 3.31-3.36 (*m*, δCH_{2b}^{Pro}), 3.48-3.53 (*m*, $CH_{2a}OH^{Leuol}$), 3.53-3.56 (*m*, $CH_{2b}OH^{Leuol}$, δCH_{2b}^{Pro}), 3.78-3.83 (*m*, δCH_{2a}^{Pro}), 3.83-3.87 (*m*, δCH_{2a}^{Pro}), 3.94-3.99 (*m*, αCH^{Leuol}), 4.00 (*d*, *J* = 8.8, αCH^{Ile}), 4.05 (*dd*, *J* = 7.8, 4.4, αCH^{Gln}), 4.15-4.20 (*m*, αCH^{Leu}), 4.18 (*d*, *J* = 8.5, αCH^{Ile}), 4.33 (*t*, *J* = 7.8, αCH^{Pro}), 4.43-4.48 (*m*, αCH^{Pro}, αCH^{Leu}).
^{13}C-NMR (75.5 MHz, CD_3OD): 10.8 (*q*, δCH_3^{Ile}), 11.1 (*q*, δCH_3^{Ile}), 15.9 (*q*, γCH_3^{Ile}), 16.3 (*q*, γCH_3^{Ile}), 21.0 (*q*, δCH_3^{Leu}), 21.1 (*q*, δCH_3^{Leu}), 22.1 (*q*, δCH_3^{Leuol}), 23.2 (*q*, CH_3^{Ac}), 23.5 (*q*, δCH_3^{Leu}), 23.7 (*q*, δCH_3^{Leu}), 23.8 (*q*, δCH_3^{Leuol}), 24.1, 24.2, 24.3 (3 *q*, 3 βCH_3^{Aib}), 25.8 (*d*, γCH^{Leu}), 26.0 (*d*, γCH^{Leu}), 26.0 (*q*, βCH_3^{Aib}), 26.1 (*d*, γCH^{Leuol}), 26.5 (*t*, γCH_2^{Pro}), 26.6 (*t*, βCH_2^{Gln}), 26.7 (*d*, γCH^{Ile}), 26.7 (*q*, βCH_3^{Aib}), 27.0 (*q*, βCH_3^{Aib}), 27.1 (*t*, γCH_2^{Pro}), 27.2 (*d*, γCH^{Ile}), 30.0 (*t*, βCH_2^{Pro}), 30.2 (*t*, βCH_2^{Pro}), 32.3 (*t*, γCH_2^{Gln}), 36.7 (*d*, βCH^{Ile}), 37.4 (*d*, βCH^{Ile}), 40.4 (*t*, βCH_2^{Leu}), 41.3 (*t*, βCH_2^{Leuol}), 50.3 (*t*, δCH_2^{Pro}), 50.6 (*t*, δCH_2^{Pro}), 51.1 (*d*, αCH^{Leuol}), 53.1 (*d*, αCH^{Leu}), 55.1 (*d*, αCH^{Leu}), 57.4 (*s*, αC^{Aib}), 57.5 (*d*, αCH^{Gln}), 57.8 (*s*, αC^{Aib}), 58.0 (*s*, αC^{Aib}), 60.1 (*d*, αCH^{Ile}), 61.4 (*d*, αCH^{Ile}), 64.3 (*d*, αCH^{Pro}), 65.2 (*d*, αCH^{Pro}), 65.8 (*t*, CH_2OH^{Leuol}).
ESI-MS (MeOH+NaI): 1212.5 (100%, $[M+Na]^+$), 617.4 (55%, $[M+2Na]^{2+}$).
Anal. calc. for $C_{59}H_{104}N_{12}O_{13} \cdot 2\ H_2O$ (MG 1225.37): C 57.82, H 8.88, N 13.71; found: C 57.75, H 8.85, N 13.81.
$[\alpha]_D^{20} = -9.0$ (*c* = 0.67, MeOH).

9.4 RP-HPLC correlation of synthetic and natural *Hypomurocins*

MS/MS Nomenclature

Many factors like the primary sequence, the amount of internal energy, the way the energy is introduced, and the charge state, influence the types of fragment ions observed in an MS/MS spectrum. *Roepstorff* and *Fohlman* [91] were the first who proposed the accepted nomenclature for these fragment ions. This nomenclature was modified by *Biemann* [92].

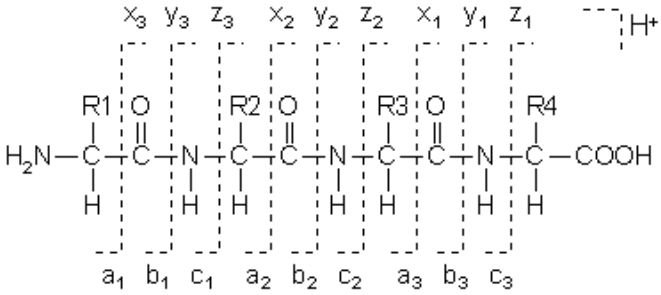


Figure 38. *MS/MS Ion Classification of Peptide Fragments*

Fragments can only be detected if they carry at least one charge. If this charge is retained on the N terminal fragment, the ion is classified as either a, b or c. If the charge is retained on the C terminus, the ion type is called either x, y or z (*Figure 38*). A subscript indicates the number of residues in the fragment.

In addition to the proton(s) at the charge d atom or group, c ions and y ions abstract an additional proton from the precursor peptide. The structures of the six mono-charged sequence ions are shown in *Figure 39*.

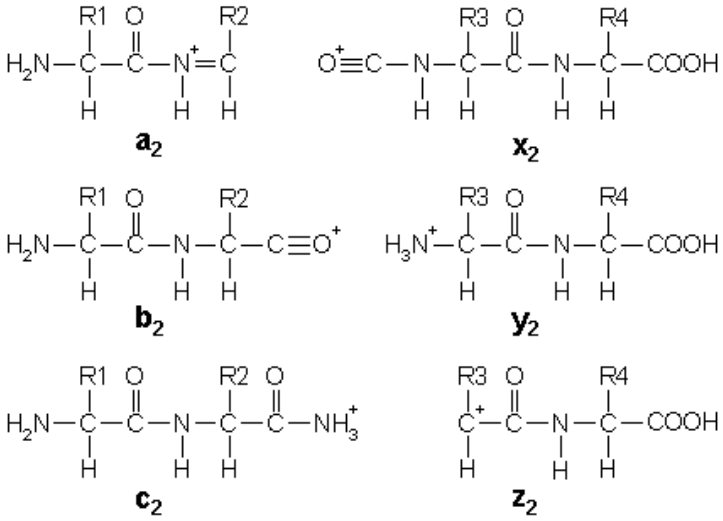


Figure 39. *Structures of the Six Monocharged Sequence Ions*

According to *Brueckner* [88], the fragments Pro^6-Leu, Pro^6-Leu-Leu, Pro^6-Leu-Leu-Aib^9, Pro^6-Leu-Ile and Pro^6-Leu-Ile-Aib^9 were labeled *PL*, *PLL*, *PLLU*, *PLI* and *PLIU*, respectively.

HM A1

HPLC-MS: t_R 28.9 min; 539.2 (100, b_5^+), 623.5 (91, y_6^+), 947.6 (8, b_9^+), 1161.5 (<1, [*M* + H]$^+$).
MS/MS (539.2): 256.1 (14, b_2^+), 327.2 (4, b_3^+ – CO), 355.2 (46, b_3^+), 454.2 (100, b_4^+).
MS/MS (623.5): 183.2 (11, [*PL* + H – CO]$^+$), 211.1 (30, [*PL* + H]$^+$), 215.2 (6, y_2^+), 279.2 (6), 296.2 (3, [*PLL* + H – CO]$^+$), 324.2 (97, [*PLL* + H]$^+$), 381.2 (6, [*PLLU* + H – CO]$^+$), 409.3 (100, [*PLLU* + H]$^+$).

HM A3

HPLC-MS: t_R 29.7 min; 215.2 (5, y_2^+), 553.3 (100, b_5^+), 623.4 (95, y_6^+), 962.5 (9, b_9^+), 1175.6 (<1, [*M* + H]$^+$).
MS/MS (553.3): 256.1 (18, b_2^+), 327.2 (4, b_3^+ – CO), 355.2 (51, b_3^+), 440.3 (<1, b_4^+ – CO), 468.2 (100, b_4^+).
MS/MS (623.4): 183.2 (12, [*PL* + H – CO]$^+$), 211.1 (32, [*PL* + H]$^+$), 215.2 (6, y_2^+), 279.2 (3), 296.2 (2, [*PLI* + H – CO]$^+$), 324.2 (71, [*PLI* + H]$^+$), 381.3 (3, [*PLIU* + H – CO]$^+$), 409.3 (100, [*PLIU* + H]$^+$).

HM A5

HPLC-MS: t_R 31.2 min; 567.4 (77, b_5^+), 623.4 (100, y_6^+), 975.5 (9, b_9^+), 1189.6 (<1, [*M* + H]$^+$).
MS/MS (567.4): 256.1 (25, b_2^+), 341.2 (4, b_3^+ – CO), 369.2 (61, b_3^+), 482.3 (100, b_4^+).
MS/MS (623.4): 183.1 (14, [*PL* + H – CO]$^+$), 211.1 (33, [*PL* + H]$^+$), 215.1 (5, y_2^+), 279.2 (8), 324.2 (98, [*PLL* + H]$^+$), 381.3 (6, [*PLLU* + H – CO]$^+$), 409.3 (100, [*PLLU* + H]$^+$).

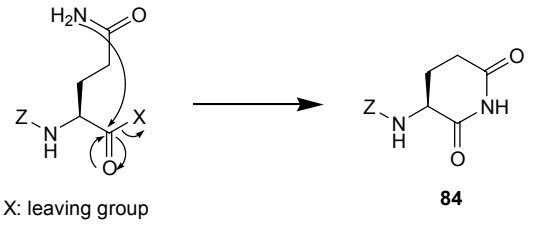
9.5 X-Ray Crystal-Structure Determination

Details of the determination of the X-ray crystal structures of compounds **6**, **33**, **38**, **47**, **60**, **68**, **69**, **72**, **76**, **77**, **79**, **82**, **84** and **85** are consigned in the database of the Institute of Organic Chemistry (OCI) of the University of Zürich (see chapter 9.1.2, X-ray crystal-structures).

In *Table 10*, the data collection and refinement parameters are listed, and views of the molecules are shown in *Figures 28*, *11*, *12*, *19*, *20*, *21*, *24*, *25*, *27*, *26*, *30*, *40* and *41*, respectively.

Finally, crystals of **84**, the cyclization product of Z-Gln-OH unit used in the experiments of this work, were obtained. They were suitable for an X-ray crystal-structure determination (*Scheme 23* and *Figure 40*).

Scheme 23. Proposed Mechanism for the Formation of **84**



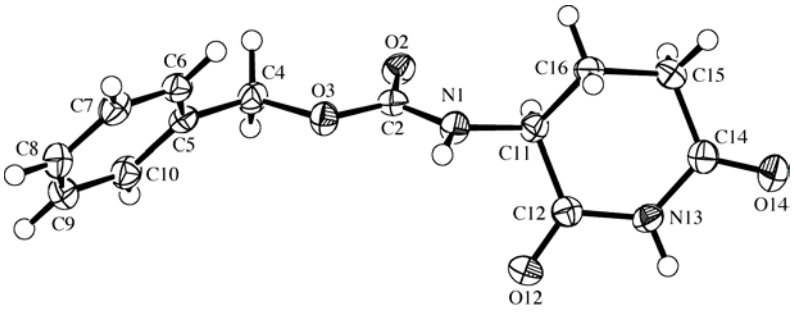


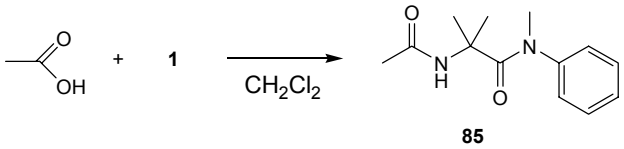
Figure 40. *ORTEP Plot* [86] *of the Molecular Structure of* **84** (50% probability ellipsoids; H-atoms given arbitrary displacement parameters for clarity)

The space group permits the compound **84** in the crystal to be enantiomerically pure, but the absolute configuration of the molecule has not been determined. The enantiomer used in the refinement was based on the expected *S*-configuration of the molecule. Intermolecular H-bonds involving both N-H groups as donors and the chain amide O-atom as the acceptor of both interactions links the molecules into infinite two-dimensional networks. Each N-H group of the molecule acts as a donor for H-bonds. The chain amide O-atom acts as the acceptor of both interactions, but these interactions come from two different neighboring molecules. The interaction involving the chain N-H group as donor links the molecules into extended chains. The interaction involving the ring N-H group as donor also links the molecules into extended chains. The

combination of both interactions links the molecules into infinite two-dimensional networks.

Compound **85**, which resulted from the reaction of acetic acid with **1**, gave also suitable crystals for an X-ray crystal-structure determination (*Scheme 24 and Figure 41*).

Scheme 24. Synthesis of **85**



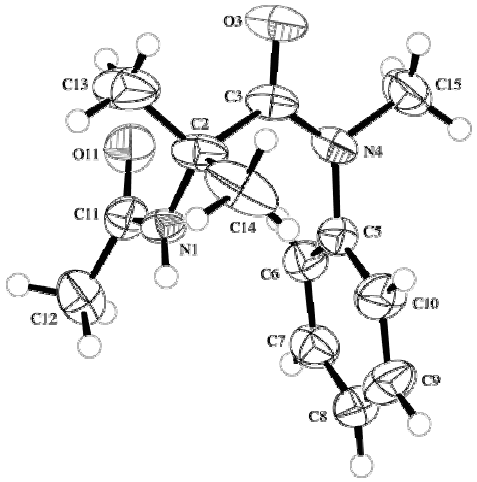


Figure 41. *ORTEP Plot* [86] *of the Molecular Structure of* **85** (50% probability ellipsoids; H-atoms given arbitrary displacement parameters for clarity)

The space group of the molecular strcture of **85** is non-centrosymmetric, but the absolute structure has not been determined and the direction of the polar axis has been chosen arbitrarily. The primary amide group forms an intermolecular H-bond with the primary amide O-atom of a neighboring molecule and thereby links the molecules into extended chains.

Table 10. *Crystallographic Data*

	6 (HG 0534)	**33** (HG 0301)
Crystallized from	H_2O/MeOH	MeOH
Empirical formula	$C_{58}H_{104}N_{12}O_{14}$	$C_5H_8N_2O_2$
Formula weight [g mol^{-1}]	1193.53	128.13
Crystal color, habit	colorless, needle	colorless, prism
Crystal dimensions [mm]	0.05 × 0.08 × 0.30	0.22 × 0.25 × 0.32
Temperature [K]	160(1)	160(1)
Crystal system	monoclinic	orthorhombic
Space group	*C*2	$P2_12_12_1$
Z	4	4
Reflections for cell determination	4995	1065
2θ range for cell determination [°]	4–46	4–60
Unit cell parameters *a* [Å]	32.8020(8)	6.4457 (2)
b [Å]	11.2610(3)	9.5398 (2)
c [Å]	19.3206(6)	9.8862 (3)
α [°]	90	90
β [°]	106.921(2)	90
γ [°]	90	90
V [Å^3]	6827.7(3)	607.91 (3)
D_x [g cm^{-3}]	1.161	1.400
μ(MoK_α) [mm^{-1}]	0.0832	0.110
Scan type	ϕ and ω	ϕ and ω
2θ(max) [°]	46	60
Transmission factors (min; max)	-	-
Total reflections measured	36755	11706
Symmetry independent reflections	5025	1051
Reflections with $I > 2\sigma(I)$	4046	879
Reflections used in refinement	5018	1051
Parameters refined; restraints	852; 163	95
Final *R(F)* [$I>2\sigma(I)$ reflections]	0.0808	0.0380
wR(F^2) (all data)	0.2316	0.1023
Weights [a; b] [1])	0.1455; 9.6387	0.0562; 0.0391
Goodness of fit	1.069	1.078
Secondary extinction coefficient	0.0025(6)	0.06 (2)
Final Δ_{max}/σ	0.001	0.001
Δρ (max; min) [e Å^{-3}]	0.82; -0.34	0.20; -0.19

[1]) $w = [\sigma^2(F_o^2) + (aP)^2 + bP]^{-1}$ where $P = (F_o^2 + 2F_c^2)/3$

Table 10 *(continued)*. *Crystallographic Data*

	38 (HG 0422)	**47** (HG 0531)
Crystallized from	$CDCl_3$	MeOH/hexane/AcOEt
Empirical formula	$C_{30}H_{35}N_3O_5$	$C_{30}H_{39}N_3O_7$
Formula weight [g mol^{-1}]	517.62	553.65
Crystal color, habit	colorless, tablet	colorless, prism
Crystal dimensions [mm]	0.05 × 0.10 × 0.22	0.15 × 0.25 × 0.33
Temperature [K]	160(1)	160(1)
Crystal system	orthorhombic	triclinic
Space group	$P2_12_12_1$	$P\bar{1}$
Z	4	1
Reflections for cell determination	2773	3287
2θ range for cell determination [°]	4 – 50	4 – 55
Unit cell parameters a [Å]	10.4178(2)	6.2705(1)
b [Å]	10.6996(2)	9.7577(2)
c [Å]	24.6636(5)	13.2491(3)
α [°]	90	106.648(1)
β [°]	90	94.273(1)
γ [°]	90	105.726(1)
V [Å^3]	2749.16(9)	737.36(3)
D_x [g cm^{-3}]	1.251	1.247
μ(MoK_α) [mm^{-1}]	0.0855	0.0888
Scan type	ϕ and ω	ϕ and ω
2θ(max) [°]	50	55
Transmission factors (min; max)	-	-
Total reflections measured	30620	17488
Symmetry independent reflections	2759	3359
Reflections with $I > 2\sigma(I)$	2388	3096
Reflections used in refinement	2757	3359
Parameters refined; restraints	356	375; 3
Final $R(F)$ [$I>2\sigma(I)$ reflections]	0.0374	0.0358
$wR(F^2)$ (all data)	0.0897	0.0852
Weights [a; b] [1])	0.0473; 0.3586	0.0367; 0.1302
Goodness of fit	1. 072	1.070
Secondary extinction coefficient	0.009(1)	0.090(9)
Final Δ_{max}/σ	0.001	0.001
Δρ (max; min) [e Å^{-3}]	0.17; -0.15	0.15; -0.16

[1]) $w = [\sigma^2(F_o^2) + (aP)^2 + bP]^{-1}$ where $P = (F_o^2 + 2F_c^2)/3$

Table 10 *(continued)*. *Crystallographic Data*

	60 (HG 0424)	**68** (HG 0430)
Crystallized from	-	MeOH
Empirical formula	$C_{10}H_{17}NO_2S$	$C_{22}H_{31}N_3O_6 \cdot H_2O \cdot MeOH$
Formula weight [g mol^{-1}]	215.31	583.56
Crystal color, habit	colorless, prism	colorless, prism
Crystal dimensions [mm]	0.15 × 0.22 × 0.35	0.35 × 0.37 × 0.45
Temperature [K]	160(1)	273(1)
Crystal system	monoclinic	monoclinic
Space group	$P2_1$	$P2_1$
Z	2	2
Reflections for cell determination	32910	2412
2θ range for cell determination [°]	4–55	4–50
Unit cell parameters a [Å]	6.6553(3)	9.7126(6)
b [Å]	9.4541(6)	11.5221(8)
c [Å]	9.2508(5)	12.2597(8)
α [°]	90	90
β [°]	103.508(3)	107.614(4)
γ [°]	90	90
V [Å^3]	565.96(5)	1307.7(2)
D_x [g cm^{-3}]	1.263	1.228
μ(MoK_α) [mm^{-1}]	0.262	0.0926
Scan type	ϕ and ω	ω
2θ(max) [°]	55	50
Transmission factors (min; max)	0.872; 0.964	-
Total reflections measured	13752	18981
Symmetry independent reflections	2568	2409
Reflections with $I > 2\sigma(I)$	2373	1762
Reflections used in refinement	2566	2407
Parameters refined; restraints	131; 1	285; 1
Final $R(F)$ [$I>2\sigma(I)$ reflections]	0.0326	0.0754
$wR(F^2)$ (all data)	0.0829	0.1861
Weights [a; b] [1])	0.0481; 0.0924	0.1224; 0
Goodness of fit	1.029	1.005
Secondary extinction coefficient	0.05(1)	-
Final Δ_{max}/σ	0.001	0.001
Δρ (max; min) [e Å^{-3}]	0.18; -0.25	0.29; -0.30

[1]) $w = [\sigma^2(F_o^2) + (aP)^2 + bP]^{-1}$ where $P = (F_o^2 + 2F_c^2)/3$

Table 10 *(continued)*. *Crystallographic Data*

	69 (HG 0458)	**72** (HG 0442)
Crystallized from	[1])	-
Empirical formula	$C_{49}H_{80}N_8O_{10}\cdot 0.5Et_2O$	$C_{41}H_{74}N_8O_8\cdot 0.5H_2O$
Formula weight [g mol^{-1}]	985.27	816.09
Crystal color, habit	colorless, prism	colorless, prism
Crystal dimensions [mm]	0.10 × 0.20 × 0.30	0.07 × 0.10 × 0.20
Temperature [K]	160(1)	160(1)
Crystal system	orthorhombic	orthorhombic
Space group	$P2_12_12_1$	$P2_12_12_1$
Z	4	4
Reflections for cell determination	5557	4550
2θ range for cell determination [°]	4–50	4–50
Unit cell parameters a [Å]	27.2779(6)	11.0876(2)
b [Å]	19.0207(4)	11.4371(2)
c [Å]	10.8797(2)	36.0079(8)
α [°]	90	90
β [°]	90	90
γ [°]	90	90
V [Å^3]	5644.9(2)	4566.2(2)
D_x [g cm^{-3}]	1.159	1.187
μ(MoK_α) [mm^{-1}]	0.0816	0.0833
Scan type	ϕ and ω	ω
2θ(max) [°]	50	50
Transmission factors (min; max)	-	-
Total reflections measured	84234	69389
Symmetry independent reflections	5539	4529
Reflections with $I > 2\sigma(I)$	4023	2559
Reflections used in refinement	5530	4525
Parameters refined; restraints	711; 114	562; 2
Final $R(F)$ [$I>2\sigma(I)$ reflections]	0.0570	0.0588
$wR(F^2)$ (all data)	0.1615	0.1565
Weights [a; b] [2])	0.0797; 2.0779	0.0699; 0.5735
Goodness of fit	1.042	1.014
Secondary extinction coefficient	0.007(1)	-
Final Δ_{max}/σ	0.001	0.002
Δρ (max; min) [e Å^{-3}]	0.26; -0.36	0.23; -0.20

[1]) AcOEt/hexane/cyclohexane/Et_2O

[2]) $w = [\sigma^2(F_o^2) + (aP)^2 + bP]^{-1}$ where $P = (F_o^2 + 2F_c^2)/3$

Table 10 *(continued)*. *Crystallographic Data*

	76 (HG 0426)	**77** (HG 0529)
Crystallized from	MeCN	[1])
Empirical formula	$C_{29}H_{46}N_4O_6$	$C_{21}H_{40.25}N_4O_{4.125}$
Formula weight [g mol^{-1}]	546.70	414.82
Crystal color, habit	colorless, tablet	colorless, prism
Crystal dimensions [mm]	0.12 × 0.15 × 0.35	0.13 × 0.25 × 0.33
Temperature [K]	160(1)	160(1)
Crystal system	tetragonal	monoclinic
Space group	$P4_3$	$C2$
Z	4	8
Reflections for cell determination	2879	5677
2θ range for cell determination [°]	4–52	4–55
Unit cell parameters a [Å]	8.9668(4)	26.9659(4)
b [Å]	8.9668(4)	12.7698(3)
c [Å]	37.304(2)	19.2335(4)
α [°]	90	90
β [°]	90	134.1707(9)
γ [°]	90	90
V [Å^3]	2999.4(2)	4750.5(2)
D_x [g cm^{-3}]	1.211	1.160
μ(MoK_α) [mm^{-1}]	0.0847	0.0806
Scan type	ϕ and ω	ϕ and ω
2θ(max) [°]	52	55
Transmission factors (min; max)	-	-
Total reflections measured	16072	53382
Symmetry independent reflections	2966	5690
Reflections with $I > 2\sigma(I)$	2481	4529
Reflections used in refinement	2964	5685
Parameters refined; restraints	375; 1	577; 51
Final $R(F)$ [$I>2\sigma(I)$ reflections]	0.0403	0.0462
$wR(F^2)$ (all data)	0.0894	0.1222
Weights [a; b] [2])	0.0359; 0.4167	0.0671; 1.3005
Goodness of fit	1.099	1.023
Secondary extinction coefficient	0.014(2)	0.0038(5)
Final Δ_{max}/σ	0.002	0.001
Δρ (max; min) [e Å^{-3}]	0.15; -0.15	0.49; -0.20

[1]) MeOH/AcOEt/hexane/CH_2Cl_2/pet. ether

[2]) $w = [\sigma^2(F_o^2) + (aP)^2 + bP]^{-1}$ where $P = (F_o^2 + 2F_c^2)/3$

Table 10 *(continued)*. *Crystallographic Data*

	79 (HG 0455)	**82** (HG 0459)
Crystallized from	-	[1])
Empirical formula	$C_{29}H_{46}N_4O_6$	$C_{54}H_{90}N_8O_{12}$
Formula weight [g mol^{-1}]	546.70	1043.35
Crystal color, habit	colorless, prism	colorless, plate
Crystal dimensions [mm]	0.15 × 0.25 × 0.33	0.02 × 0.20 × 0.20
Temperature [K]	160(1)	160(1)
Crystal system	monoclinic	monoclinic
Space group	$P2_1$	$P2_1$
Z	4	4
Reflections for cell determination	5633	10599
2θ range for cell determination [°]	5–50	4–50
Unit cell parameters a [Å]	13.0906(6)	10.8603(2)
b [Å]	11.7084(5)	27.0371(5)
c [Å]	20.0843(8)	20.5452(4)
α [°]	90	90
β [°]	91.218(3)	102.967(1)
γ [°]	90	90
V [Å^3]	3077.6(2)	5878.9(2)
D_x [g cm^{-3}]	1.180	1.179
μ(MoK_α) [mm^{-1}]	0.0825	0.0832
Scan type	ω	ϕ and ω
2θ(max) [°]	50	50
Transmission factors (min; max)	-	-
Total reflections measured	33105	95928
Symmetry independent reflections	5652	10587
Reflections with $I > 2\sigma(I)$	4627	6978
Reflections used in refinement	5652	10578
Parameters refined; restraints	852; 421	1409; 304
Final $R(F)$ [$I>2\sigma(I)$ reflections]	0.0978	0.0663
$wR(F^2)$ (all data)	0.3009	0.1895
Weights [a; b] [2])	0.2; 0	0.1280; 0
Goodness of fit	1.294	0.961
Secondary extinction coefficient	0.013(5)	-
Final Δ_{max}/σ	0.011	0.001
Δρ (max; min) [e Å^{-3}]	0.40; -0.35	0.44; -0.29

[1]) Et_2O/MeOH/AcOEt/hexane/cyclohexane

[2]) $w = [\sigma^2(F_o^2) + (aP)^2 + bP]^{-1}$ where $P = (F_o^2 + 2F_c^2)/3$

Table 10 *(continued)*. *Crystallographic Data*

	84 (HG 0352)	**85** (HG 0530)
Crystallized from	CD_3OD	MeOH
Empirical formula	$C_{13}H_{14}N_2O_4$	$C_{13}H_{18}N_2O_2$
Formula weight [g mol^{-1}]	262.26	234.30
Crystal color, habit	colorless, tablet	colorless, plate
Crystal dimensions [mm]	0.07 × 0.25 × 0.27	0.05 × 0.15 × 0.30
Temperature [K]	160(1)	160(1)
Crystal system	orthorhombic	monoclinic
Space group	$P2_12_12_1$	*Cc*
Z	4	4
Reflections for cell determination	2098	1392
2θ range for cell determination [°]	4−60	4−52
Unit cell parameters *a* [Å]	5.7466(1)	14.0195(5)
b [Å]	8.7276(2)	9.8942(3)
c [Å]	24.3618(5)	9.7724(3)
α [°]	90	90
β [°]	90	98.262(2)
γ [°]	90	90
V [Å^3]	1221.84(4)	1341.48(8)
D_x [g cm^{-3}]	1.426	1.160
μ(MoK_α) [mm^{-1}]	0.107	0.0789
Scan type	ϕ and ω	ω
2θ(max) [°]	60	52
Transmission factors (min; max)	-	-
Total reflections measured	21560	10426
Symmetry independent reflections	2081	1312
Reflections with $I > 2\sigma(I)$	1786	1187
Reflections used in refinement	2079	1312
Parameters refined; restraints	181	163; 2
Final *R(F)* [$I>2\sigma(I)$ reflections]	0.0372	0.0366
wR(F^2) (all data)	0.0926	0.0944
Weights [a; b] [1])	0.0446; 0.1237	0.0533; 0.3271
Goodness of fit	1.046	1.038
Secondary extinction coefficient	0.022(5)	0.024(3)
Final Δ_{max}/σ	0.001	0.001
Δρ (max; min) [e Å^{-3}]	0.19; -0.18	0.13; -0.11

[1]) $w = [\sigma^2(F_o^2) + (aP)^2 + bP]^{-1}$ where $P = (F_o^2 + 2F_c^2)/3$

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